



Floral Biology and Interspecific Crossability of *Litsea salicifolia*, a Host Plant of the Muga Silkworm

R. Ravi Kumara

CSB - Muga P-3 Seed Station, Kobulong 798615, Nagaland, India

Abstract

The study aimed to examine the floral biology and interspecific crossability of *Litsea salicifolia*, a secondary host plant of the Muga silkworm (*Antheraea assamensis* Helfer). Observations focused on sex expression, anthesis, stigma receptivity, pollinator activity and fruiting ecology. Controlled hybridization with *L. monopetala* was successful, resulting in a 14.12% seed set. These findings suggest the potential for improving leaf yield and palatability for the Muga silkworm through interspecific hybridization in *Litsea salicifolia*.

Keywords: *Dighloti*, floral biology, interspecific crossability, *Litsea salicifolia*, *L. monopetala*, Muga silkworm.

Introduction

The Muga silkworm (*Antheraea assamensis* Helfer) is an endemic sericigenous species found in northeastern India. Due to its golden-colored silk, it holds a distinct position in the global sericulture map. India produces approximately 250 MT of Muga silk annually, and in 2023-24, it produced around 252 MT of Muga raw silk (CSB, 2024). This species is multivoltine and polyphagous, feeding on a wide range of host plants, with 36 species reported so far (Kumara *et al.*, 2024). Among them, *Persea bombycina* (Som) and *Litsea monopetala* (Soalu) are the primary host plants, while *Litsea salicifolia* (Dighloti) and *Litsea citrata* (Mejankori) are considered secondary. Sometimes, *L. salicifolia* is used as a primary food plant for both commercial and seed cocoon production (Barah and Sengupta, 1991; Gogoi, 1999). During winter, *L. salicifolia* can replace *L. monopetala* to cope with the defoliation and poor leaf quality of the latter. Traditional silkworm farmers feed *L. salicifolia* leaves to weak Muga larvae to restore their vigor and strength. Muga larvae fed on *L. salicifolia* during the first and second instars and then transferred to Som or Soalu leaves produce healthier, disease-free cocoons (Gogoi, 1999; Mech *et al.*, 2015). Several

bioactive compounds in *L. salicifolia* have antimicrobial properties, which may protect the silkworm larvae from pathogens (Wang *et al.*, 2016). Its leaf biochemical content at various maturity stages—such as moisture (61.24–72.21%), total minerals (4.45–8.53%), crude fiber (5.47–23.45%), reducing sugars (1.11–2.14 mg/g), total soluble sugars (3.47–5.17%), starch (7.69–11.58%), chlorophyll a (1.39 mg/g), chlorophyll b (1.06 mg/g), β -sitosterol (0.38–0.69%), total phenols (31.10–101.97 mg/g), ascorbic acid (0.58–1.58%), chlorogenic acid (0.05–0.34%), and tannins (3.07–5.00%)—is comparable to that of primary host plants (Neog *et al.*, 2013). The plant can be propagated through both sexual (seeds) and asexual methods (air layering, stem cuttings, leaf node cuttings, and apical bud cuttings) (Ram *et al.*, 1993). A mature plant can yield approximately 7 kg of leaves (Bhuinya and Mukherjee, 2011). The plant's bushy nature, good pruning response, and ease of rearing supervision and management make it particularly suitable for Muga silkworm rearing (Ram *et al.*, 1993; Gogoi, 1999).

Litsea salicifolia (Roxb. ex Nees) Hook. f. (2n=2x=24), belonging to the Lauraceae family

(Mehra and Bawa, 1968) and locally known as Dighloti in Assamese, is found in tropical and subtropical countries such as India, China, and Japan (Gangopadhyay *et al.*, 2019). It is distributed across the sub-Himalayan tract from Nepal eastward, ascending up to 6,000 feet above sea level. Its range includes Assam, the Garo and Khasi Hills of Meghalaya, the Naga Hills of Nagaland, the Bengal plains, the Sundarbans, Chittagong in Bangladesh, and parts of Myanmar (Srivastav *et al.*, 2005). This plant is a small evergreen, dioecious tree or shrub that grows up to 10 meters tall. The branchlets are glabrous, and the stem is minutely hairy, slender, and horizontal. The bark is grayish or chocolate brown, about 0.3 cm thick, with a yellowish blaze that turns dark brown with age. The leaves of *L. salicifolia* are simple, elliptic to linear-lanceolate in shape, and either acute or acuminate at the tip. They are glabrescent (almost hairless) on the upper surface and glaucous (bluish or grayish-green) and pubescent (covered with fine hair) on the underside. The leaves are chartaceous (papery) and entire (with smooth edges), with a reticulate venation. The lateral nerves number 10-15 pairs, and the petiole measures 0.7-2.5 cm in length. The leaf blade measures 8.0-25.0 cm in length and 3.0-5.0 cm in width, and the plant is exstipulate, meaning it lacks stipules (Hooker, 1885). Leaf venation includes pinnate primary veins, with secondary veins that increase in spacing toward the base and display a uniform angle. There are no intersecondary veins. The tertiary veins are a mix of opposite percurrent and alternate percurrent, sinuous and acute. Quaternary and quinary veins form a regular polygonal reticulate pattern, with well-developed areolation and unbranched freely ending veinlets, typically excurrent and looped. No marginal teeth are present. Unicellular, unbranched trichomes are more abundant on the abaxial (underside) surface than on the adaxial (upper) surface (Jalil *et al.*, 2024). Six morphotypes of *L. salicifolia* have been reported, classified as varieties based on leaf morphology: ellipsoidea, elongata, attenuata, laurifolia, polyneura, and reticulata (Hooker, 1885).

In addition to its importance in sericulture, *L. salicifolia* has significant medicinal value. Extracts from its bark, fruits, and leaves have been traditionally used to treat chronic inflammation-related conditions, including asthma and for wound healing (Gangopadhyay *et al.*, 2019). The plant also contains essential oils with strong antioxidant and insect-repellent properties (Noosidum *et al.*, 2008; Ko *et al.*, 2010). Major bioactive compounds identified in *L. salicifolia* include mesembrine, gallic acid, asperulosid, quercetin-3-O-glucoside, isoorientin, barbaloin, eudesmanolide, capsaicin, kaempferide, and brucine—all of which have been reported to possess potent antioxidant and anti-inflammatory properties (Puppala *et al.*, 2023). In the present study, an attempt was made to cross *L. salicifolia* with *L. monopetala*, while also examining its floral biological characteristics.

Materials and Methods

Study Site and Plant Material

The study was conducted during the flowering and fruiting seasons (March–July) of 2024 at the CSB – Muga P-3 Seed Station, located in the Naga Hills of the Kobulong Block, Mokokchung district, Nagaland, India (26.4131° N latitude, 94.5329° E longitude, and at an altitude of approximately 4,000 feet above sea level). This site represents a natural habitat of *Litsea salicifolia*, where mature (5-year-old), healthy individuals were selected for detailed investigations. Additionally, *Litsea monopetala* was used in interspecific hybridization experiments to assess crossability.

Floral Biology Observations

During the flowering season, thirty inflorescences were randomly collected from fifteen mature *L. salicifolia* trees in field to determine the floral sexual status. Based on floral morphology, trees were classified as either staminate (male) or pistillate (female), and the sex ratio was subsequently calculated. Flowering phenology was recorded using a total of 20 trees (both staminate and pistillate), each of which was individually tagged and monitored throughout the flowering period to

document the date of first and last flowering, thereby defining the duration of blooming for each tree. Reproductive output was assessed by recording the production rate of involucre buds (umbels), the number of florets per umbel, and the number of stamens or stamens per floret in both male and female trees. The presence and production rate of nectar glands in florets of both flower types were also documented. Anthesis and anther dehiscence were monitored by tagging and observing ten involucre umbels each from staminate and pistillate trees. Pollen output and stigma receptivity were assessed following standard protocols described by Dafni *et al.* (2005). Pollen viability was assessed using acetocarmine staining.

Insect Pollinator Studies

Insect visitation and pollination behavior were recorded through direct observations of involucre umbels on both staminate and pistillate trees. Field observations were conducted during peak flowering over four consecutive days. Insects visiting the umbels were recorded every hour from morning (06:00 am) to evening (06:00 pm), with each observation session lasting 15 minutes. These data were used to calculate the hourly and overall percentage of foraging visits, as well as the contribution of each insect category to the total visitation frequency. The observed insects were further categorized by functional group (e.g., pollinators vs. nectar thieves) to assess their potential role in pollination.

Controlled Pollination and Crossability Tests

Interspecific hybridization was performed using five-year-old pistillate trees of *L. salicifolia* as the female parent. Flower buds were bagged at the pre-anthesis stage using butter paper bags to prevent unwanted pollination. Each bag enclosed 10–20 umbels. Pollen was collected from the male parent, *L.*

monopetala, at the peak of anther dehiscence and was manually applied to receptive stigmas using a fine brush. A total of 450 flowers were pollinated. Unpollinated flowers served as controls to evaluate autonomous fruit set. Seed set was determined as the percentage of pollinated flowers that developed into mature fruits.

Results and Discussion

Phenology

The reproductive phenology of *L. salicifolia* offers valuable insights into its ecological adaptation and reproductive strategy in the Naga Hills, India. The species exhibits a dioecious reproductive system (Fig. 1a), with distinct staminate and pistillate individuals, and a skewed sex ratio favoring males at approximately 3:1. Such a disparity may influence pollen availability and, consequently, reproductive success, a pattern commonly observed in dioecious taxa where male-biased populations can enhance pollination efficiency (Barrett and Hough, 2013). The onset of flowering at three years of age in both sexes indicates a relatively early reproductive maturity, which may be advantageous in disturbed or dynamic environments where rapid reproduction ensures persistence (Oñate and Munné-Bosch, 2009). Flower bud initiation begins in February, with anthesis spanning from early March to May and peaking in the third week of March. This synchronization with late winter to early spring conditions likely corresponds to optimal climatic and biotic factors such as temperature, moisture availability, and pollinator activity (Alburquerque *et al.*, 2004). The inflorescences are flat-topped, grey, pubescent pseudo-umbels borne in large numbers along the branchlets, at the axils of leaves, and at the apices of the branchlets (Fig.1b & Fig.1c).



Figure 1. Morphological view showing: *Litsea salicifolia* (a); Twig with staminate umbels (b); Twig with staminate flowers (c)

Staminate flower morphology and biology

The morphology and reproductive biology of staminate flowers in *L. salicifolia* provide important insights into the species' pollination strategy and floral specialization. Each umbel comprises 1 to 8 pedicellate involucral buds in both male and female trees. The involucral bud encloses 4 to 7 florets. The florets, borne on 2–4 mm long pedicels, are pale yellow, slightly foetid, actinomorphic, and measure approximately 3.5 ± 1.2 mm in length and 4.2 ± 1.3 mm in width (Fig.2a) with a slight foetid odor—traits often associated with generalist pollination syndromes, possibly targeting small insects such as flies or beetles (Ramana *et al.*, 2019). A notable feature of the staminate flower is the inconsistency in perianth development. The presence of perianth lobes only in peripheral florets suggests a differentiation in floral roles within a single inflorescence, possibly enhancing visual or olfactory cues for pollinators while economizing structural investment in centrally positioned florets (Simpson, 2010). The villose texture and creamy white coloration of the perianth lobes, when present, may also aid in attracting pollinators by providing tactile or visual stimuli (Zhang *et al.*, 2014).

The androecium is highly variable, with 10 to 16 (average 14 ± 1.2) stamens arranged in 2–3 whorls, displaying a gradation in filament length that may improve pollen presentation by spatially structuring pollen release across the floral surface (Sun *et al.*, 2024). The villose filaments, each potentially bearing 0 to 2 short-stalked fleshy glands, further highlight the complexity of floral architecture (Simpson, 2010). These glands could play a role in secreting substances that attract or reward pollinators (Zhang *et al.*, 2014). The wide variation in gland number (2–14 per floret) suggests individual or developmental plasticity, which may contribute to reproductive success under diverse environmental conditions (Albuquerque *et al.*, 2004). Anther morphology and dehiscence mechanisms are also noteworthy. The four-celled, introrse, semi-orbicular anthers open through a specialized valvular mechanism, ensuring gradual pollen release (Fig.2b). This mode of dehiscence, occurring between 08:00 and 12:00 hours, likely aligns with peak activity periods of local pollinators. The partial retention of pollen within the anther locules may also function as a secondary pollen presentation mechanism, potentially enhancing pollen transfer efficiency over extended periods (Sun *et al.*, 2024). The pollen fertility was 82.36 ± 4.23 percent (Fig. 2c). The

presence of a green, glabrous pistillode with a well-developed but ovule-less ovary is indicative of floral homology and evolutionary remnants of bisexual ancestry.

This vestigial structure could also contribute to floral symmetry or potentially act as a tactile cue for pollinators (Simpson, 2010).

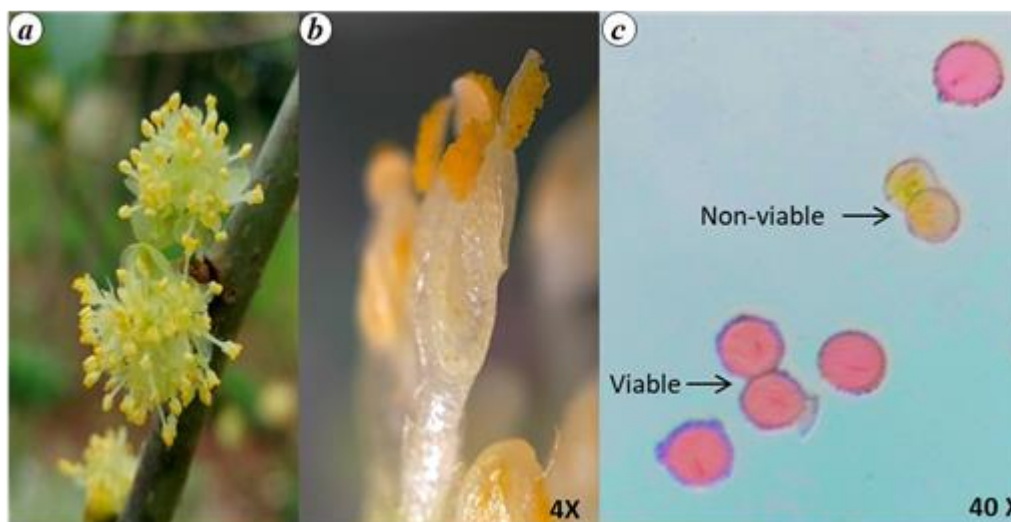


Figure 2. Morphological view showing: Male flowers (a); Stamen (b); Pollen grains (c)

Pistillate flower morphology and biology

The pistillate (female) floral morphology of *L. salicifolia* reflects a high degree of structural and functional specialization that likely enhances reproductive success in its native montane habitat. The florets are borne on 2–3 mm long pedicels, are pale yellow and mildly foetid, are consistent in appearance with the staminate flowers (Fig.3a), suggesting that both sexes attract similar pollinators—likely small, scent-oriented insects (Ramana *et al.*, 2019). The actinomorphic symmetry and relatively larger floret size (5.1 ± 0.8 mm in length and 4.7 ± 0.5 mm in width) compared to staminate florets may improve visibility and accessibility for pollinators (Creux *et al.*, 2021). A remarkable feature shared with male flowers is the irregular distribution of perianth lobes, with their presence largely restricted to peripheral florets. This may indicate a strategic resource allocation, wherein more conspicuous floral traits are localized to the most visible positions within the inflorescence, serving to enhance pollinator attraction while minimizing energetic costs (Graham, 2016).

The presence of staminodes—sterile structures morphologically similar to

stamens—further supports the idea of ancestral bisexuality and may function as a mimicry system to enhance pollinator visitation (Simpson, 2010). These staminodes, creamy white and villose, are arranged in three concentric whorls and vary in number from 12 to 22 (mean: 17.2 ± 1.3). Their decreasing length from outer to inner whorls mirrors the pattern seen in staminate flowers, possibly contributing to floral symmetry and guiding pollinators toward the functional stigma (Graham, 2016). Importantly, the glandular bases of the staminodes secrete nectar, which serves as a reward for visiting insects (Zhang *et al.*, 2014). The total number of glands per floret ranges from 6 to 17. The pistil is green, glabrous, and 4.6 ± 0.7 mm long (range: 3–6 mm). The nectar glands—swollen, stalked pads located at the base of the staminodes—undergo a highly specialized secretory process. Cuticle degradation and epidermal disintegration prior to anthesis allow nectar to be actively secreted and presented as glistening droplets, suggesting strong visual and possibly olfactory cues for pollinators (Escalante-Pérez and Heil, 2012). The structural adaptation of a villose base enclosing the glands likely serves a dual role: aiding in nectar retention and offering

protection against desiccation or microbial contamination (Simpson, 2010). The ovary is ovoid, unilocular, and contains a single ovule. The style is filiform, 2.7 ± 0.5 mm long (range: 2–4 mm), and topped with a peltate, slightly bifid, papillate, wet, and shiny stigma that extends above the height of the staminodes (Fig.3b). The filiform style and peltate stigma—elevated above the staminodes—are

finely structured for effective pollen capture (Zhang *et al.*, 2014). The wet, papillate stigma surface, along with its receptivity extending up to three days post-anthesis, indicates a prolonged window for successful fertilization. This may be particularly advantageous in dioecious species where synchrony between male and female flowering is critical (Barrett and Hough, 2013).

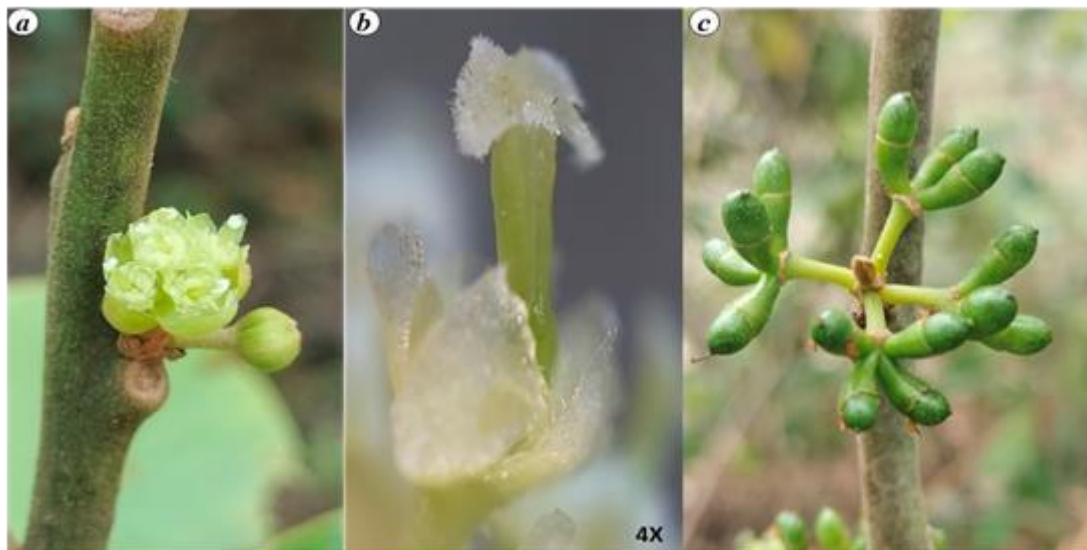


Figure3. Morphological view showing: Female flower (a); Pistil (b); Young fruits (c)

Insect foraging activity and pollination

The diurnal insect foraging patterns observed in *L. salicifolia* highlight a generalized pollination system involving a diverse assemblage of insect visitors. Both staminate and pistillate umbels attract a similar spectrum of foragers, including bees (*Apis cerana*, *A. florea*, *Trigona iridipennis*), wasps (*Vespa bicincta*), flies (*Chrysomya megacephala*, *Eristalinus arvorum*, *Sarcophaga* sp.), and butterflies (*Rapala iarbus*, *Hasora chromus*), indicating floral traits that appeal broadly across taxonomic groups (Ramana *et al.*, 2019). Among these, *Apis* species and *Eristalinus arvorum* were the most consistent and regular visitors, collecting both pollen and nectar, thereby positioning them as the primary effective pollinators. Their foraging behavior—targeting both floral resources—facilitates direct contact with reproductive organs, thus increasing the likelihood of successful pollen transfer (Zhang *et al.*, 2014). The remaining insect groups, although

consistent in presence, foraged only for nectar and did not consistently contact the anthers or stigmas. Nonetheless, they still contribute to pollination through incidental pollen transport, as evidenced by the presence of pollen on their bodies (Wappler *et al.*, 2015). Temporal foraging patterns show clear alignment with floral biology. Foraging activity begins around 07:00 am, peaks at midday—coinciding with the timing of anther dehiscence in male flowers and stigma receptivity in females—and tapers off by evening 5:30 pm. This synchrony maximizes pollination efficiency by ensuring overlap between pollinator activity and floral reproductive readiness. Bees dominated visitation rates, accounting for nearly half of the daily foraging activity (48%), followed by flies (26%), wasps (14%), and butterflies (12%). These proportions further emphasize the central role of hymenopterans and dipterans in *L. salicifolia* pollination (Ramana *et al.*, 2019).

Fruiting ecology

The fruiting ecology of *L. salicifolia* reveals key aspects of its reproductive output, dispersal strategy, and recruitment success in its native forest ecosystem. The production of 1–14 fruits per involucre flower cluster (Fig.3c), though numerically modest, reflects the reproductive limitation common in dioecious and insect-pollinated species, where pollination success is highly contingent on effective pollen transfer (Barrett and Hough, 2013). Fruit maturation begins in the last week of May and continues through July. The most frequent fruit output of only 1–4 fruits (Fig.4 a) per umbel further emphasizes the resource investment and pollination-dependence of successful fruit development. Natural fruit set variation (15.36% to 40.27%) among individuals likely reflects variability in pollinator activity, floral display, or microhabitat conditions (Alburquerque *et al.*, 2004). In contrast, no fruit set was observed in the unpollinated control flowers. On average, the seed setting rate was 23.39%. The uniform presence of a single ovule per pistillate floret and the consistent match between fruit set and seed set indicate a tightly regulated reproductive system with minimal post-fertilization abortion (Simpson, 2010). The rapid maturation of the fruits within four weeks may reflect an adaptive strategy to avoid pre-dispersal seed predation and synchronize fruit availability with the activity of key frugivores (Chatterjee and Basu, 2015).

Fruits of *L. salicifolia* are fleshy, non-endospermic, and exhibit traits typical of zoochorous species: attractive coloration (ripening from green to purple/black), a distinctive aroma when crushed, and a glossy appearance—all features likely evolved to

attract vertebrate dispersers (Tiffney, 2004). The fruits mature in approximately 30 days and are one-seeded, non-endospermic, fleshy berries measuring 10–11 × 5–6 mm, borne on pedicels 4–7 mm long. The average fruit weight is 0.46 ± 1.51 g (Fig. 4b). The seeds are brown with irregular light grey bands on the surface, measuring 8–9 × 3–4 mm, with an average weight of 0.17 g (Fig. 4c). Morphologically, the fruits and seeds are similar to those of *L. monopetala* (Bhuinya and Mukherjee, 2011). The involvement of large-bodied frugivores such as squirrels (*Ratufa* spp.) and hornbills (*Buceros* spp.) underscores a specialized mutualism, wherein these animals act as both consumers and long-distance seed dispersers (Chatterjee and Basu, 2015). The behavior of transporting fruits before consumption promotes seed shadow dispersal—displacing seeds away from the parent plant and reducing competition, density-dependent mortality, and pathogen pressure near maternal trees (Nathan and Casagrandi, 2004). Interestingly, seeds excreted by these animals remain intact, albeit softened, suggesting that endozoochory may aid in seed coat weakening and facilitate germination, though germination still appears strongly season-dependent (Chatterjee and Basu, 2015). In the natural setting, germination is restricted to the rainy season, indicating a response to soil moisture availability, which is crucial for non-endospermic seeds lacking stored nutritional reserves. Despite effective dispersal mechanisms, the germination rate remains relatively low (~22%), which may be attributed to seed predation, microbial attack, or unsuitable microsite conditions (Euler *et al.*, 2014).



Figure 4. Morphological view showing: Fruiting twig (a); Mature fruits (b); Seeds (c)

Interspecific crossability

In sericulture, interspecific hybridization has been performed in silkworm host plants such as mulberry (*Morus* spp.), the sole host plant of *Bombyx mori* (Das and Krishnaswami, 1965), and *Terminalia arjuna* and *Terminalia tomentosa*, primary host plants of *Antheraea mylitta* (Gargi *et al.*, 2015). Building on these earlier efforts, interspecific crossability studies on *L. salicifolia* have demonstrated its reproductive compatibility with *L. monopetala*, indicating the potential for gene flow and hybrid formation within the genus *Litsea*. Controlled pollination of *L. salicifolia* with *L. monopetala* resulted in a successful seed set of 14.12%. This outcome suggests that the two taxa may share similar floral structures, synchronized phenology, or compatible pollen-pistil interactions, facilitating fertilization and early seed development (Ferriol *et al.*, 2023). The success of this cross may also reflect evolutionary proximity or historical gene exchange between these species in sympatric habitats (Chapman and Goring, 2010). However, further studies are needed to assess the viability, germinability, and fertility of the resulting hybrid seeds, along with their phenotypic characteristics and suitability for silkworm rearing.

Conclusion

The study on *Litsea salicifolia* provided valuable insights into its floral biology, pollination ecology, and interspecific crossability. Distinct floral traits and sexual dimorphism were documented, with insect pollinators—particularly bees—playing a crucial role in facilitating reproductive success. The successful interspecific crosses with *L. monopetala* indicate promising prospects for the genetic improvement and conservation of host plant diversity in Muga sericulture. These findings enhance our understanding of reproductive biology in dioecious tree species and offer practical implications for sustainable silkworm rearing and effective host plant management.

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Source of support: Nil;

Conflict of interest: The authors declare no conflict of interests.

Cite this article as:

Kumara, R.R. "Floral Biology and Interspecific Crossability of *Litsea salicifolia*, a Host Plant of the Muga Silkworm." *Annals of Plant Sciences*. 14.06 (2025): pp. 6853-6862.