

Pollination Biology and Breeding Systems of *Calophyllum apetalum* Willd. from Western Ghats of Karnataka

¹ C.N. PRASANNA KUMAR, ² R.K. SOMASHEKAR, ³ B.C. NAGARAJA, AND ⁴ D. SHIVAPRASAD

Department of Environmental Science, Bangalore University, Bangalore 560056, Karnataka, India.

² Corresponding author; Email: rksmadhu@gmail.com

¹ Email: prasannakumar348@gmail.com; ³ Email: nagenvi@gmail.com; ⁴ Email: shivaprasad.gkvk@gmail.com

ABSTRACT

Flower morphology, anthesis, pollen production, *in-vitro* pollen germination, stigma receptivity, and foraging nature of floral visitors of *Calophyllum apetalum* Willd. (family Clusiaceae) have been studied across the sites of Western Ghats of India. The flowers are bisexual, relatively small with numerous stamens, open in early and late morning, pollinated by both anemophily and entomophily that helps effective pollination and results in successful fruit set. The highest and lowest mean fruit set were observed with manual hand cross (94.8±8.93%) and open pollination (73.2±14.16%) respectively. The manual cross and self-pollinations are significantly different ($\alpha=0.05$, $p\leq 0.01$) indicating the importance of naturally mediated cross pollination by pollinators. The high index of self-incompatibility (>0.25) specifies that the species is self-compatible. Emasculation followed by bagging did not yield any fruit set thereby showing the absence of apomixis.

Key Words: Phenology; Pollination; Breeding Systems; Western Ghats.

INTRODUCTION

The pollination and reproductive biology of several tropical plant species inhabiting tropical forests have been extensively studied (Momose et al. 1998, Johnson and Steiner 2000, Oliveira and Gibbs 2000, Nayak and Davidar 2010) but the information on pollination and reproductive biology of many tree species from the rainforests of tropical Western Ghats is limited (Devy and Davidar 2003). The genus *Calophyllum* belongs to the family Clusiaceae, which consists of about 130 species of which seven are reported from India (Joshi et al. 2013). *Calophyllum apetalum* Willd. is a multi-purpose evergreen tall tree, endemic to the Western Ghats of southern India (Nair and Seeni 2003), distributed along the banks and streams with an elevation range up to 1300 m (CSIR 1992), and often shows stem buttresses. Different parts of this plant are used in the traditional system of medicine for the treatment of several diseases (Joshi et al. 2013). Seed oil is reported

to possess antiseptic properties and is used in the treatment of rheumatism, leprosy, scabies and other cutaneous afflictions while the leaf juice is used for gastric trouble (CSIR 1992). The chemical examination of different parts of *C. apetalum* led to the isolation of xanthenes and tri-terpenoids (Govindachari et al. 1967, Iinuma et al. 1997, Xiao et al. 2008). The stem bark exudes a translucent resinous fluid used as vulnerary, resolutive and anodyne (Chopra et al. 1956). The timber is good for construction of boats and furniture and, is suitable for plywood. Species of *Calophyllum* are gaining importance as a source of anti-HIV medicines (Daniels and Patil 1995, Cesar et al. 2011). During the colonial rule, *C. apetalum* timber was extracted from its known habitat for shipbuilding. Though felling of this tree is no longer practiced, plant number has declined presumably due to several biotic and abiotic pressures. Seeds have a low germination capacity (40%) and the oily seeds lose their viability quickly. Moreover, the fruits ripen during the rainy season and this plant being

a riparian species, the seeds are often lost in floods, further limiting the reproduction (Cruden 1977).

A few studies within the rain forests of Western Ghats have attempted to rise the seedlings of the species in the disturbed sites under introduced plantations (Nagaraja et al. 2001a 2001b). In spite of its ethnobotanical and economic importance with widespread distribution, no information is available on its pollination and reproductive biology. The present study was carried out to discern floral phenology, pollination biology and breeding systems of *C. apetalum* inhabiting the evergreen forests of Western Ghats, Karnataka. Besides it also focuses on the relationship with pollinators. The current study precedes our understanding of floral and pollination biology of *C. apetalum*, which has not been emphasized and this information would help in the conservation of its genetic diversity.

STUDY AREA

The study was carried out at lowland tropical forests of Agumbe region within the Someshwara wild life sanctuary of Western Ghats, Karnataka, classified as tropical wet evergreen forests, rich in *Dipterocarpus indicus* - *Humboldtia brunonis* - *Poeciloneuron indicum* association (Champion and Seth 1968, Pascal et al. 1988, Padaki and Parthasarathy 2000). The study sites lie at 13°30'9.64"N and 75°05'25.15"E with elevation ranging from 400 to 600 m above mean sea level, and harbor rich endemic flora (Pascal et al. 1988). The mean annual rainfall is between 7000 and 7500 mm, and mean annual temperature is 22 °C (Padaki and Parthasarathy 2000).

MATERIALS AND METHODS

The phenological events were studied by randomly selecting 10 trees from undisturbed sites of Western Ghats. The observations were made on different phenophases includes (i) leaf emergence and drop (ii) flowering and anthesis and (iii) fruit setting and dispersal during the period January to December 2013. The phenological observations were made every week during the period of high activity (flowering season) and continued until fruit maturation and dispersal with three week intervals during the rest of the year. The floral phenology of the species was studied by tagging five inflorescences each on 10 randomly selected individual trees and the flowering stages were observed since bud

initiation until senescence. A particular phenophase was considered to have started when 20% of individuals were observed in that particular phase, and was considered to have been completed when less than 20% individuals remained in the same phase without any change (Sharma and Khanduri 2007, Prasannakumar et al. 2013)

The flowers with different stages, including the unopened matured buds were selected and tested regularly for the stigma receptivity using peroxidase test (Galen and Plowright 1987, Dafni and Marcia 1998) and observed until flower senescence using 10x hand lenses. Flowers were monitored at every three hour interval to determine the timing of anther dehiscence and pollen release. The *in-vitro* pollen germination and viability was studied using Brew-Baker media and 2,3,5-triphenyl-tetrazolium chloride (TTC) solution (0.5%) respectively (Shivanna and Rangaswamy 1992). The pollen count was made by taking 15 randomly selected anthers from 10 randomly selected flowers using haemocytometer (Nanda et al. 2006) and the numbers of ovules were counted by taking the cross section of ovary (Cruden 1977, Pias and Guitian 2001).

Pollination and Breeding Systems

The breeding experiments were carried out on ten matured mother trees. Five inflorescences in each case per tree were selected for assessing the fruit set through open pollination, hand self-pollination, hand-cross pollination, and one time pollinator visitation and for apomixis. The selected inflorescences were tagged and left open for pollination by natural means and the efficacy of open pollination was confirmed upon fruit set. Manual hand pollination experiments included autogamy, xenogamy performed by counting the number of flowers on inflorescence followed by emasculation, manual pollen transfer to the stigmatic surface followed by bagging with a nylon mesh cloth bag to avoid any external pollination (Duffy et al. 2009). Apomixis was observed by emasculating the anthers followed by bagging without permitting further pollination (Baum 1995). The efficiency of fruit set upon pollinator visit was assessed by bagging the flowers immediately after one time visit by at least any one of the pollinator species (Nayak and Davidar 2010). The pollinators were observed throughout the day particularly when their activity is high between 06:00-18:00 hours and the frequency of their visitation was quantified on 20 randomly selected inflorescences. The pollinators were differentiated from floral visitors by observing their type

of foraging activity and landing sites (Fenster et al. 2004, Prasannakumar et al. 2013).

Statistical Analysis

Variations were compared by using paired Student's *t*-test for comparing the mean percentage of fruit set, by considering open pollination as control, against treatments such as manual hand self-pollination, hand cross pollination, and one time pollinator visitation. The data were analyzed using the statistical packages PAST (Hammer et al. 2001) and MegaStat (Orris 2003).

RESULTS

Phenology and Floral Biology

The peak leaf fall was observed during the last week of July and it continued till second week of October when the fresh leaf emerged. The flowering started during the last week of October and continued till the third week of November and fruit onset began by the end of November. The average number of days taken by the flowers to mature is 13-15 after initiation. The peak flower anthesis occurred between 08:15-9:30 hours. Fruit set initiation was observed 2-3 days after pollination and fertilization. The leaves of *Calophyllum apetalum* were pale red during the flush season (November– February) up to around 1–3 weeks; pinkish-red between 3rd and 5th

week; pale green during 5th to 8th week and dark green after 8 to 10 weeks. Flowers are in axillary racemes, white, bisexual, incomplete and actinomorphic; Sepals 4, bi-seriate and free, petals absent; Stamens numerous, connate basally, anthers introse, tetra-sporangiate, open by longitudinal slits, connective with glands; Gynoecium unilocular with a single ovule; basal placentation with a peltate stigma.

Pollen Production, Germination and Viability

In-vitro pollen germination was studied using Brew Baker media and viability using different concentrations of TTC solution ranging from 0.1-0.5%. The mean percent pollen germination in Brew baker media was found to be 14.15 ± 8.03 (mean \pm sd) while the mean percentage of pollen viability in 0.4% TTC solution as 88.24 ± 2.43 (Mean \pm sd). The mean pollen production per flower is estimated to be around 148.8×10^4 . The fluorescent microscopic studies confirmed mean pollen viability as 92.16 ± 6.36 percent. The flowers only produced pollen as a pollination reward and do not produce any nectar.

Breeding Systems

Breeding experiments (Figure 1) revealed the highest mean fruit set through hand cross pollination (94.82 ± 8.93) and the lowest with open pollination (73.19 ± 14.6). The paired *t*-test between natural (open) against

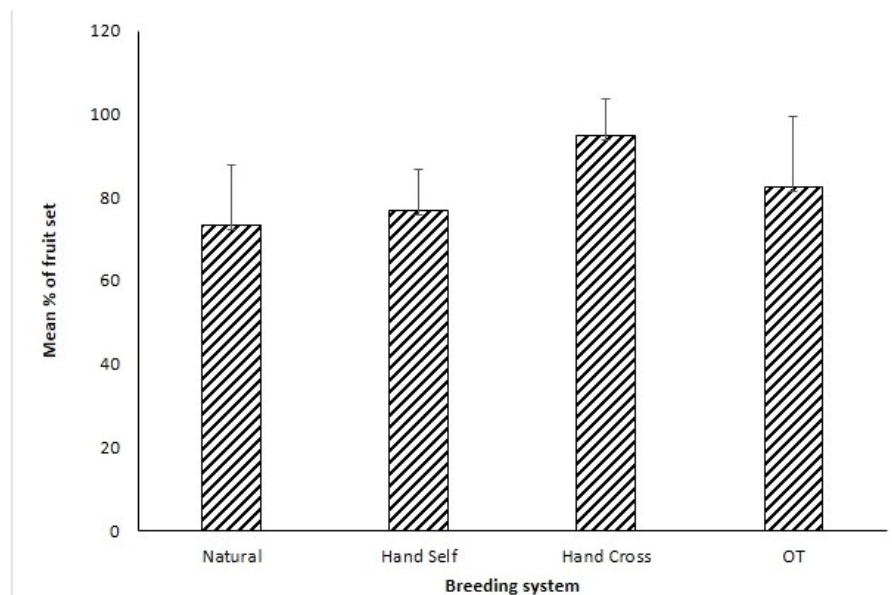


Figure 1. Mean fruit set with different manual breeding experiments

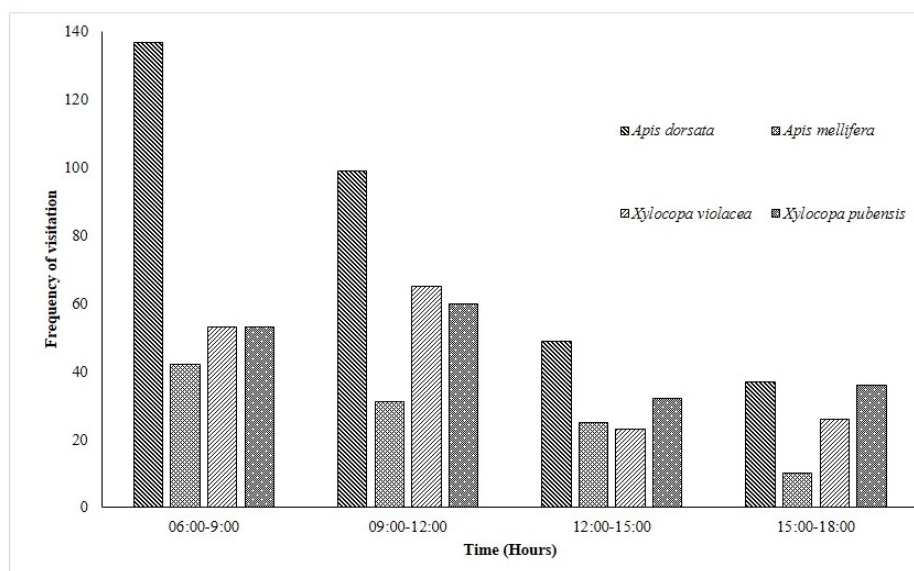


Figure 2. Pollinator visitation frequency of *Calophyllum apetalum*

hand cross pollination and hand self-pollination with hand-cross pollination were significantly different ($N=10$, $CI=95\%$, $p \leq 0.01$). An insignificant relationship was observed between Fruit set by natural pollination with one time pollinator visit and self-pollination ($N=10$, $CI=95\%$, $p \geq 0.5$). The species with an index of self-incompatibility ratio, (ISI) >0.25 confirms it as self-compatible. The major Pollinators of the species included honeybees (*Apis dorsata*, *A. mellifera*) and carpenter bees (*Xylocopa violacea* and *X. pubescens*) with high frequency of visitation throughout the day (Figure 2).

DISCUSSION

The species is facing a high threat status mainly influenced by unscientific extraction of high quality timber. Also, problem associated with the is regeneration patters is another threat since the species distributed along the riparian habitat and most of the seeds get flooded and a few of them that settle on the river banks constitute saplings. These are also subjected to intense pressure during rainy season and chance of getting washed away is more. Chopping of branches of even tender trees for producing organic compost has worsened the problem. Late rains sometimes lead to floral abortion as experienced during the present work. Improper and variable flowering period does not ensure proper and

fruit set and regeneration. There is also threat in the form of floral pests, pre-mature fruit abortion etc. The results of breeding experiments showed insignificant difference between natural pollination with manual self and pollination upon one time pollinator visit suggesting that most of the flowers visited by pollinators facilitates self-pollination. It is also supported by an abundance of pollen. The flowers are homogamous with less out-crossing index (Cruden 1977) suggesting that they prefer fruit set with self-pollination. Nonetheless, considerably good pollination and fruit set in this species also suggest pollination occurring through anemophily. The very position of the anthers surrounding the stigmatic surface as an outer whorl ensures the dispersal of pollen. The highest fruit set through manual hand crossing experiments suggest the possibility that the species also prefers selectively cross pollination. The morphology of the flowers itself suggest it is most attentively pollinated self-pollination. The blooming of flowers in a single tree almost synchronizes with bright white tepals attracts more pollinating individuals. Canopy trees of the same species ordinarily flower all together, i.e., mass flower (Ashton 1988, Appanah 1990), and a large quantity of floral reward is efficiently harvested by the eusocial bees, which have a communication system to recruit their colony members onto the flowers. This is partly caused by the migration of pollinators from the fringes of the forest to forage on the superabundance of flowers. The flowers of most of the Clusiaceae offer either rich nectar,

pollen, or resin as rewards for pollinators (Gustafsson and Bittrich 2002) and the higher pollen production in each flower of *C. apetalum* invites the attention of innumerable pollinators that contribute to successful fruit set even with onetime pollinator visit.

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