Article

Reproductive biology of *Dipterocarpus indicus* Bedd.- An endangered species from Western Ghats, India

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Abstract
The *Dipterocarpus indicus* Bedd. is one among the dominant tropical evergreen tree species of the family Dipterocarpaceae, that grows upto a height of 37m or more. The tree is popularly known for timber and oleoresin and, it is one of the major habitat for the critically endangered lion-tailed macaques, among the other associated tree species such as *Diospyros candolleana, Holigorna grahamii, and Parsea macrantha.* The tree is endemic to Western Ghats region of India with a threat status as endangered at global level. The flowering is irregular, but mass flowering occurs once in every 4-7 years. The flower initiation was observed from last week of December 2012 and continued until first week of January 2015. The flowers are borne on conspicuous drooping axillary racemes. The flowers are hermaphrodite, actinomorphic and complete. Corolla with a twisted aestivation, showing five white petals bears a pink strip in the middle. The peak flower anthesis observed between the 08:00 to 10:00 hours. The pollen production was quantified to be 264.78±59.21 and 12420±59.21 per anther and flower respectively. Ovary is superior, syncarpous, with three locules each containing two ovules arranged in axile placentation. The observations on pollinators revealed that no biotic vectors are involved in pollination and the flowers are adopted to wind pollination mechanism. Breeding experiments revealed that the flowers are self-compatible and pollinated by means of both self and cross pollination. The manual cross pollination yielded highest fruit set (69.53±1.77%), followed by manual self-pollination (59.77±2.88%) and open pollination (56.95±2.98%). The paired *t*-test between manual cross and self-pollination, and open pollination with manual cross were significant ($p \leq 0.05$) revealing the importance of pollinator species in bringing out successful fruit set. The controlled experiments also revealed the absence of apomixis. The natural self-pollination and less abundance or the absence of pollinators is likely to lead to inbreeding depression and impulse it more prone towards critically endangered category.

Keywords reproductive biology; pollination; breeding system; endanger species; fruit set.

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1 Introduction

The family Dipterocarpaceae, is well known for their timber species. It consists of 19 genera and around 580 or more species (Londono et al., 1995; Morton, 1995). Most of the dipterocarps are distributed over the tropical belt of three continents of Asia, Africa and South America (Maury-Lechon G and Curtet, 1998). *Dipterocarpus indicus* is found in the evergreen forests of Southern Western Ghats from North Kanara southwards all along the Western Ghats down to Kerala and Tirunelveli and Kanyakumari districts of Tamil Nadu. The tree is endemic in the West Coast tropical evergreen forests (Champion and Seth, 1968; Ravikumar et al., 2000). In Karnataka, it is observed in the wet evergreen forest of Chikkamagaluru, Dakshina Kannada, Hassan, Kodagu, Shivamogga and Uttara Kannada forests (Ravikumar et al., 2000). The tree species is popularly known for its timber and oleoresin and it is one of the major habitat for the critically endangered lion-tailed macaques, among the other associated tree species such as *Diospyros candolleana, Holigorna grahamii, and Parsea macrantha.* At present the Dipterocarp dominates the international tropical timber market because of its high wood quality. Oleoresin is used in the preparation of spirit, oil varnishes and lithographic inks. It is also used as a adulterant of dammar and as an remidy for rheumatism (Maury-Lechon and Curtet, 1998). The current threat status of *Dipterocarpus indicus* is endangered at global level (Ashton, 1998). Keeping this in view, the present study focused on phenology, pollination and breeding system of *D. indicus*.

2 Study Area

The study was carried out in the Agumbe region of Someshwara Wildlife Sanctuary, situated in Udupi-Shivamogga districts within the central Western Ghats, Karnataka. These forests are classified as tropical wet evergreen forest, dominated with *Dipterocarpus indicus-Humboldita brunonis-Poeciloneuron indicum* and other associated species (Champion and seth, 1968; Padaki and Parthasarthy, 2000). Agumbe region falls within 13^{o}30’9.64”N and 75^{o}5’25.15”E with an elevation range of 400-600 m above mean sea level (MSL). These forests are composed of rich endemic flora (Pascal et al., 1988). Agumbe is one of the wettest regions in Karnataka, with a mean annual rainfall between 5000 to 8000 mm.

3 Material and Methods

3.1 Vegetative and reproductive phenology

The phenological events were studied by selecting 25 mother trees, marked randomly from the study location. The observations were made on phenophases such as (1) leaf sprouting and maturation, (2) flowering and anthesis, (3) fruiting, and (4) leaf and fruit drop, for a period of four consecutive years from January 2011 to January 2015. The phenological records were made every week during the high activity period of flowering season from October to March, till fruit maturation. The observations were continued on other phenophases with three week interval during the rest of the year as suggested by Kumar et al. (2013).

3.2 Floral biology

The study pertaining to floral biology was initiated from the very beginning of floral bud initiation. The inflorescences were selected and marked on matured mother trees to observe the flowering period and different stages of floral development. Observations were continued until fruit formation. The time of anthesis was noted. To observe the dehiscence of anther and stigma receptivity, hand lens (10×) was used before and after the opening of flowers (Tidke and Thorat, 2011). Pollen morphology of *D. indicus* was studied by acetolysis technique as described by Shivanna and Tandon (2014).

3.3 Pollen production, germination, viability, and pollen-ovule ratio

Pollen production was determined from randomly selected matured anthers taken from flower buds (Nair and Rastogi, 1963; Nanda et al., 2006), for the three consequent flowering seasons between November 2011 and December 2013. The number of ovules was counted by taking a cross section of the ovary (Cruden, 1977).

In-vitro pollen germination studies were carried out using Breawbaker media. Freshly dehisced pollen grains were placed in requisite concentration of sugar following "Hanging Drop Technique" (Brewbaker and Kwack, 1963). A pinch of boric acid was added to each concentration to facilitate initiation of germination. The pollen viability was assessed by aniline blue fluorescence microscopy and by 0.5% acetocarmine solution; the stainability was taken as an index of viability as described by Shivanna and Rangaswamy (1992).

3.4 Floral visitor's dynamics and behavior

Pollinators were observed over 12 hours each day during flowering period, particularly between 06:00-18:00 hrs. and the time spent by each pollinator and floral visitors was noted (Fenster et al., 2004).

The behavior of insect visitors during flowering period was observed at different hours of the day, at each study site. The observations were also made on their mode of approach, the type of forage they collect, contact established with the essential organs of the flowers and the activities of the forager during the visits. Number of flowers visited per bout by floral visitors and the time spent on each flower were also noted (Tidke and Thorat, 2011).

3.5 Breeding studies

Breeding experiments were carried out manually by hand pollination of the flowers as briefed here under

I. Apomixis: mature flowers were selected from the inflorescence before anthesis and emasculation was carried out followed by bagging.

II. Autogamy: matured flowers were selected from the tagged inflorescences and bagged.

III. Allogamy: matured flowers were selected from the tagged inflorescences emasculated before anthesis and pollen grains from a mature flower of another plant was deposited on the receptive stigma.

IV. Natural pollination: Mature flowers from the inflorescence were marked and observed for the pollination.

To study the developmental stages of anther and ovary, the flowers of different developmental stages were collected randomly from the mother trees and flowers were fixed in FAA (5 ml acetic acid, 5 ml formalin and 90 ml 50 % ethyl alcohol). After customary methods of dehydration and paraffin embedding (Johansen, 1940) the sections of floral buds and pistils, were cut at 6-13 microns stained using safranin.

4 Results and Discussion

4.1 Vegetative and reproductive phenology

The leaf initiation started during second week of December. The young leaf initiation starts after the fall of stipule. The colour of the stipule remained pink/red and the initiated young leaves appeared light green during the last week of December. The flower bud initiation was observed during third week of December and continued till the end of January 2013 and 2014. The peak flowering was observed during second week of January (Shivaprasad et al., 2014). Comparativetly flower production was very less during 2013-2014 and massive during 2011. However, during 2011-2012 and 2013-2014 most of the flowers aborted (premature fall) between the period from the flower initiation to fruit maturation. There was no flowering during 2012 and 2014. Also there was no fruit set during 2013-2014 (Fig. 1). Similar, observations have been recorded in *Vateria indica*, a member of Dipterocarpaceae, which failed to produce reproductive parts during two years, Sundarapandian et al. (2005) reported that *Hopea parviflora*, *Dimocarpus longon* and *Hydnocarpus alpine* produce flowers and fruits once in two years. Wycherley (1973) reported that occasional flowering with an interval of about 3-8 years is more common in Dipterocarps. The general belief is that dipterocarp trees flower in 5 to 6 year intervals, but the actual interval is irregular and erratic. Furthermore, the timing of flowering and

fruiting depends on the species and location. As such it is difficult to predict the flowering attitude of dipterocarp trees. However, in a good flowering year, most dipterocarp tend to flower gregariously (Suzuki, 2006). Premature fall of flowers and fruits is probably due to irregular and scanty precipitation during flowering and fruiting season. Rain drops not only wash away the major part of the meager amount of pollen produced, but also break down a very large number of buds, flowers and developing fruits.

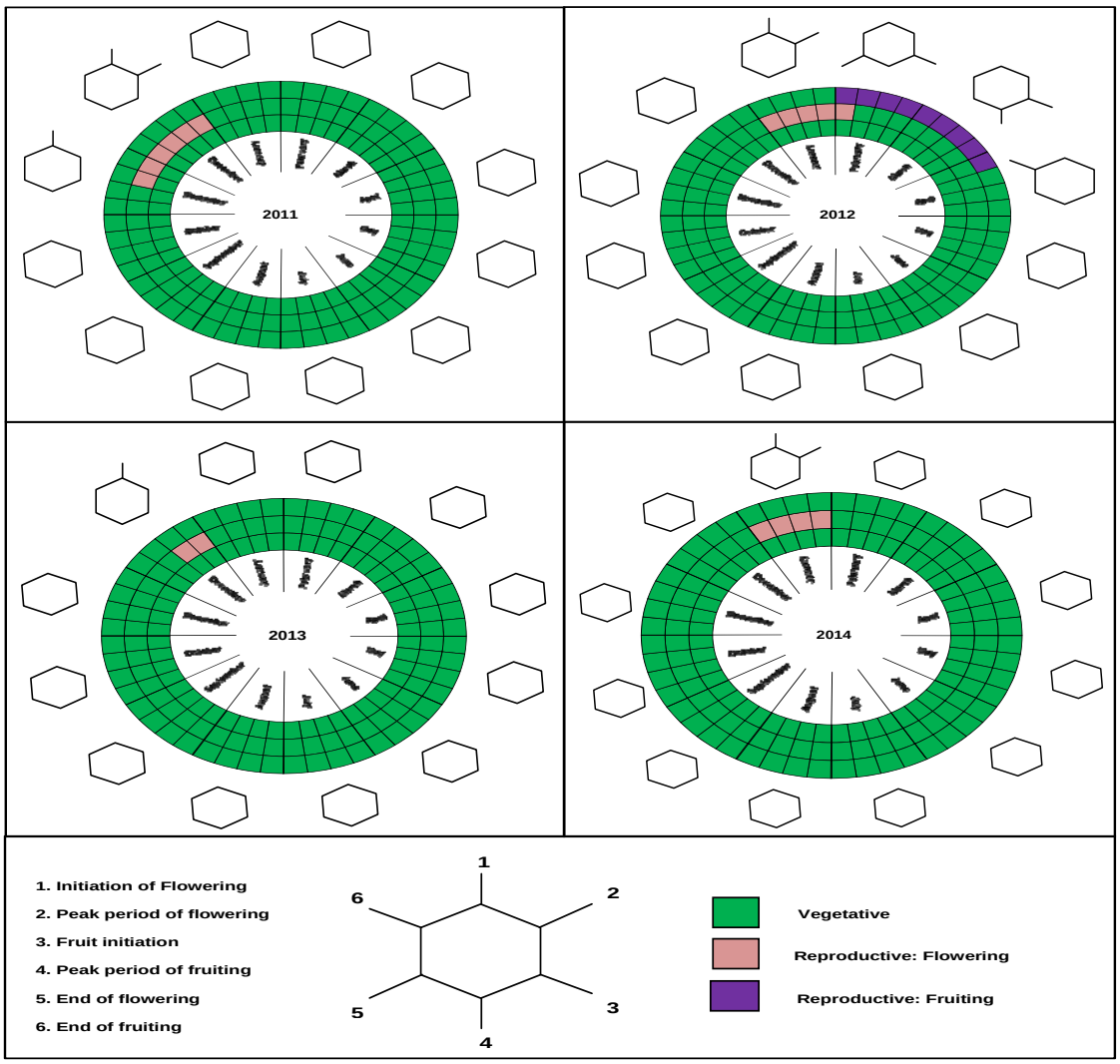

Fig. 1 Phenological events of *Dipterocarpus indicus* during 2011 to 2014.

4.2 Floral biology

Flowers of *D. indicus* are complete, bisexual, actinomorphic, pentamerous in nature, calyx are five, sepals fused with valvate aestivation green and corolla with five free petals, have twisted aestivation white with pink strip in the middle. The average number of anther per flower is 30, arranged compactly in three whorls. The average length of the anther is 7.63±0.10 mm (mean±S.D). The ovary is superior, syncarpous, with three locules each containing two anatropous, bitegmic, crassincellate ovules. Style is relatively long, with erect slender style and measured about 11.47±1.06 mm (mean±S.D) in length. The pollens of *D. indicus* are monad with radial symmetry, 3-colpate, elliptic or circular in shape (Fig. 2). Similar observations on pollen morphology have been recorded in *D. indicus* (Vasanthi and Grard, 2007).

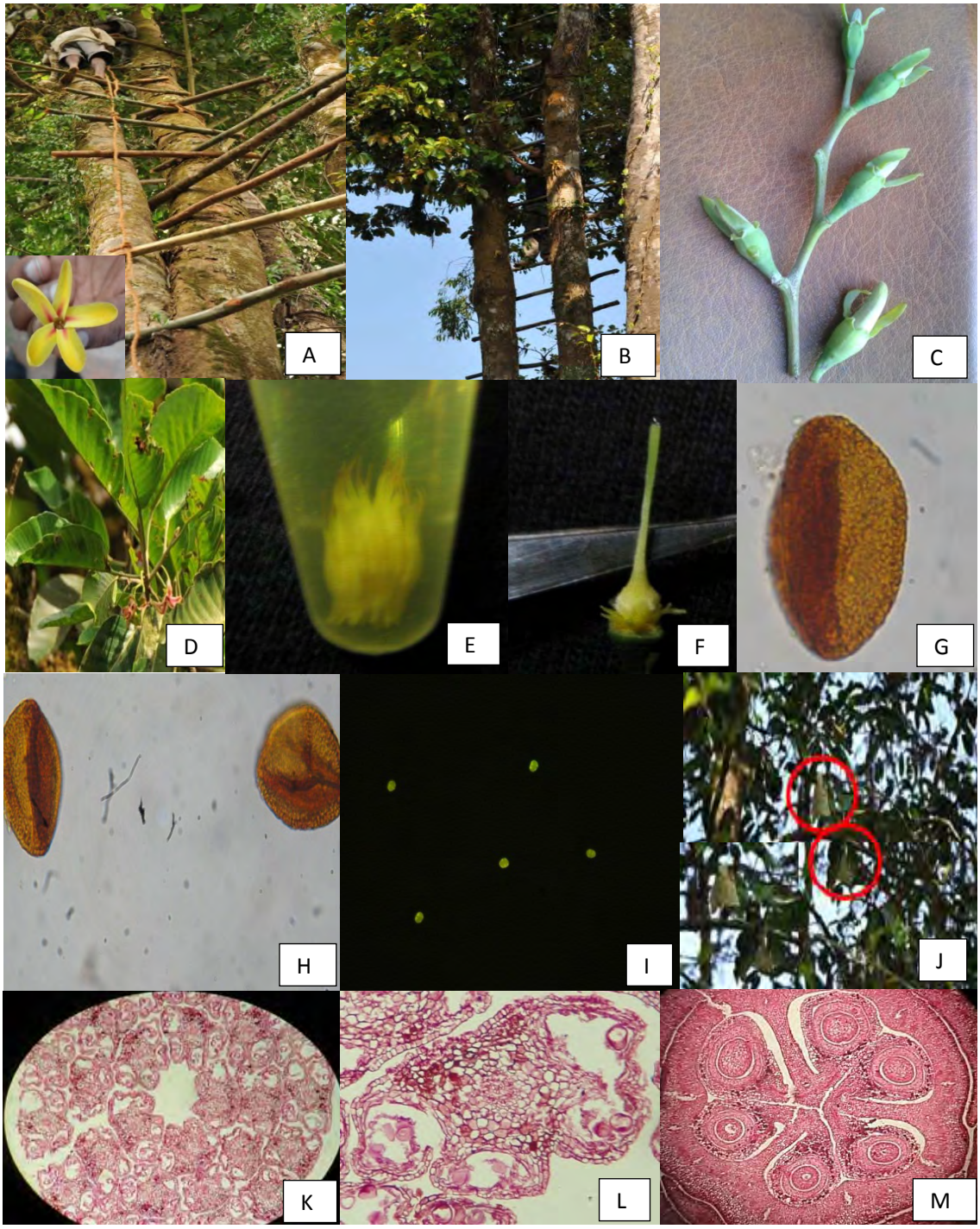

Fig. 2 A) Ladder preparation B) Pollinator observation and flower collection C) Inflorescence D) Floral anthesis E) Stamens collection F) Stigma receptivity test G & H) Acetolysis test for pollen I) Pollen viability test J) Bagged inflorescences K) Stamen section L) Anther section M) Ovary section.

4.3 Pollen production, germination, viability and pollen-ovule ratio

The average number of pollen grains per anther ranged from 520 to 1200 and the average is 742 grains, and per flower it was 22260±5426.27 grains during 2011-2012, it ranged between 200 and 960 with an average of 548 pollens per anther, and per flower it was 16440±6108.22 pollen during 2013-2014. Percentage pollen

germination is 52.64±13.41% (Mean±S.D) in Brewbaker media. The average pollen viability is 62.94% and 57.68% during during 2011 and 2013 respectively. The pollen:ovule ratio was 3710 in 2011 and 2740 in 2013. Similar observations on pollen production, viability, germination and pollen-ovule ratio is recorded in another threatened tree species, *Madhuca longifolia* (Tidke and Thorat, 2011) and Kumar et al. (2013) in *Madhuca neriifolia* and Shivaprasad et al. (2015) in *Cinnamomum sulphuratum.* Pollen to ovule (P/O) ratio variation is attributable to the efficiency of pollination system. Cruden et al. (1977) observed increased P/O ratios from self to cross pollinated crops and argued that the latter are less efficient in pollination and hence they produce more pollen grains. Similarly, Shankar and Ganeshaiah (1982 and 1984) attributed the greater P/O ratios in the wild species over their domesticated species to greater risks of pollination encountered by the wild species.

Table 1 Pollen production and Pollen – ovule ratio of *Dipterocarpus indicus* during 2011 to 2014.

Year	Total pollen production per flower	No. of ovule	Pollen ovule ratio
2011	22260	6	3710
2012		No flowering	
2013	16440	6	2740
2014		No flowering	

4.4 Pollinator observation and breeding systems

Flowers of *D. indicus* are pollinated by wind. Floral structure, pollination syndromes and the observations on pollinators of the flowers in *D. indicus* revealed that no biotic vectors is responsible for pollinating the flowers. The flowers have adopted to a wind pollination syndrome only. High altitude tree species possess dull-coloured flowers with no specialized in their floral structure. Sharma et al. (1999) reported that dull colour flowers of *Boswellia serrate* attracted a few pollinators. Earlier studies of pollination mechanisms in tropical tree species documented wind-pollination in *Shorea robusta* (Atluri et al., 2004) *Madhuca indica* (Reddi, 1976; Kuruvilla, 1989), *Mimusops elengi* (Reddi and Atluri, 1981), *Cotoneaster distichus* and *Emblica officinalis* (Burd and Allen, 1988) from south India. However, Trigona species was the major pollinator for one of the four dominates dipterocarp species, *Shorea siamensis* in dry deciduous dipterocarpus forests of Thiland (Ghazoul and McLeish, 2001).

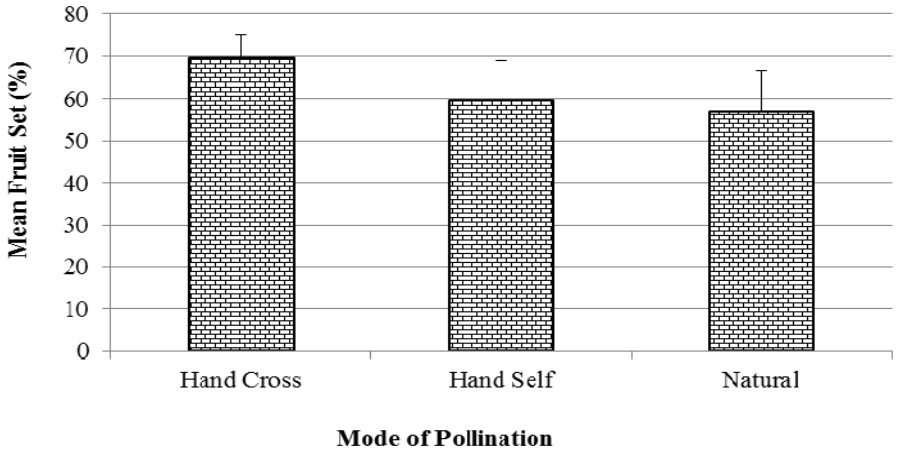

Fig. 3 Breeding study of *D. indicus* during 2011.

Breeding experiments revealed that the flowers are self-compatible and pollinated by means of both self and cross pollination. The manual cross pollination yields highest fruit set (69.53±1.77%), followed by manual self-pollination (59.77±2.88%) and open pollination (56.95±2.98%). The paired *t*-test between manual cross and self-pollination, and natural pollination with manual cross were significant ($p \leq 0.05$) revealed the importance of pollination in successful fruit set. The controlled experiments also revealed the absence of apomixes (Fig. 3). Population size and plant density are closely associated with the attraction and activity of pollinators. Small populations are less attractive to pollinators, and the reduction in population size results in decreased fruit or seed production because of insufficient pollen transfer (Agren, 1996). *Dipterocarpus indicus* occur in small populations due to fragmentation and this has lead to inbreeding depression and floral abortion. The population size of pollinators in the forest fluctuates depending on the magnitude of general flowering (Nagamitsu, 1998). The low density of trees in stands reduces the inter-tree movement of pollens, promotes self-pollination and culminating in low outcrossing rate as observed in *D. indicus*. Nearly the same situation is true in *Shorea trapezifolia, Stemonoporus oblongifolius, Shorea congestiflora* and *S. megistophylla* (Murawski et al., 1994a, b).

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