

Pollination Ecology and Breeding System of *Impatiens trichocarpa* Hook. f. (Balsaminaceae): An Endemic Balsam of Western Ghats

S. K. KULLOLI AND A. K. SREEKALA*

Plant Systematics and Evolutionary Science Division, Tropical Botanic Garden and Research Institute, Pacha-Palode, Trivandrum 695 562, Kerala, India

*Corresponding author; E-mail: shreekulloli@gmail.com

ABSTRACT

A study of floral phenology, pollen production, foraging nature of flower visitors and breeding systems were studied in *Impatiens trichocarpa* Hook. f. an endemic balsam of Western Ghats. The flowers are beautifully coloured and open in the night and continue up to morning. Anther dehisced one day before anthesis which confirmed the protandrous condition of the flower. Stigmas are receptive at the time of anthesis and remains so far 20 hour. Flowers offer pollen and nectar to the visitors. Honey bees, hawk moths, butterflies, flies etc. are the major pollinators of *Impatiens trichocarpa*. Manual pollinations revealed that the species permits geitonogamous and xenogamous type of pollinations. The percentage of fruit set in manually pollinated flowers is higher than that resulting from open pollination. Its high altitude habitat, narrow environmental niche, fragmentation of populations, low percentage of seed germination and floral damage caused by the insect could be the reasons for its limited distribution in the wild.

Key Words: Anthesis, Pollination, Stigma Receptivity, Western Ghats, Xenogamy.

INTRODUCTION

Impatiens L. (Balsaminaceae) is phytogeographically an unique genus which has its greatest development in the Indian region and is found chiefly in moist sub-temperate areas. It is essentially a sub-cosmopolitan genus of the old world being distributed mainly in the mountains of tropical Asia and Africa. It is also located in Madagascar, Philippines, China, Java, Sumatra and Sri Lanka (Bhaskar 1981). In India, the genus is represented by 205 species, mainly distributed in Western Himalayas. Hills of North eastern states and Western Ghats. In Peninsular India, there are 92 species of which 80 are remarkably endemic and confined to Western Ghats. Due to their restricted distribution, nearly 30 species are already in threatened category (Vajravelu and Daniel 1983, Pandurangan and Pushpangadan 1997). Though the ideal climatic conditions prevailing in the Western Ghats region provide suitable habitat for the balsams, their population is rapidly declining due to various factors such as habitat degradation, fragmentation of popula-

tion, anthropogenic pressures, reproductive constraints etc. A comprehensive study on pollination biology of rare and endemic balsams has not so far been made due to their habitat specificity, delicate structure and explosive fruits. Successful pollination is an essential pre-requisite for survival of plants in natural communities and for obtaining better yield of plants. Variability is controlled by the breeding system of which pollination mechanism forms an integral component (Bhattacharyya and Mandal 2000). Against this background, one such endemic balsam namely, *Impatiens trichocarpa* has been selected for the investigation on phenology, pollination, breeding systems etc., to find out possible reasons for its limited distribution in the Western Ghats.

MATERIALS AND METHODS

Impatiens trichocarpa Hook. f. was collected from Devikulam of Munnar, Idukki district, Kerala and Mukurti National Park, Nilgiri, Tamil Nadu. The

plants were multiplied and grown in the garden for detailed study. It is a herbaceous balsam and may attain a height of 2 m. Five populations of 20 plants each were selected in natural habitat and observations made on day to day basis during flowering season on flower initiation, development, anthesis, post-pollination events etc. Floral morphology was studied with the help of hand lens and dissection microscope. Pollen fertility and viability were assessed by acetocarmine glycerin staining technique, and by FCR test using Fluresein diacetate. Pollen ovule ratio was worked out as per the method suggested by Cruden (1977). To study the pollen germination *in vitro*, pollen grains collected from fresh flowers were incubated in sucrose medium of different concentrations (2, 5, 10, 15, and 20%) and Brewbakers medium. After one hour, the percentage of pollen germination and tube elongation were noted. Stigma receptivity was observed with the help of hand lens. *In vivo* pollen germination studies were conducted as per the method suggested by Joshirao and Saoji (1989).

Continuous observations during July 2005-January 2007 were made on behavior of different pollinators. The pollinators were collected and identified with the help of experts. The type of food collected by different visitors and the daily foraging period were recorded by close observations. Different pollination systems such as Autogamous self pollination (T1), Geitonogamy (T2), Xenogamy (T3) and Natural pollination (T) were conducted in the field. Treatment 'T2' was conducted to examine geitonogamy through artificial pollination using pollens from different flowers of the same plant. Treatment 'T3' was done to examine xenogamy through artificial cross pollination using pollens from flowers of different plants but within the populations. Twenty healthy flowers for each treatment within the population were chosen randomly. After 25-30 days fruits and seeds were collected and their numbers noted. The weight of each seed and percentage of seed germination were also recorded.

RESULTS AND DISCUSSION

Impatiens trichocarpa Hook. f. starts flowering from the third week of July and extended up to January with maximum bloom in September (Table 1, Figure 1a). The flower buds take 8-12 days from initiation to full bloom. The plants were in flower for a total of 180 days in a year and the average life span of each flower

Table 1. Floral characters of *Impatiens trichocarpa*

Floral Characters	Observations
Inflorescence	Axillary raceme
Flower type	Hermaphrodite, zygomorphic
Flower colour	Pale pink
Flowering period	July-January
Odour	Mild
Nectar	Present (4.8 ± 0.72 µl)
Flower opening time	02.00-03.30 hr
No. of anthers/flower	5
Anther dehiscence time	0230-0330h (one day before anthesis)
Anther dehiscence mode	Apical slit
Average no. of pollens/anther	7656 ± 30
Mean no. of pollen grains/flower	38,280 ± 50
Mean no. of ovules/flower	20
Pollen-ovule ratio	1914:1
Pollen type	3-colporate
Pollen size	32.72 µm
Pollen shape	Spherical
Stigma type	Wet and non-papillate

is 2-3 days. The Flowers bloom in the night between 0200-0300h and remain fresh till the morning. Bhaskar and Razi (1974) had reported that majority of wild balsams grown in the high altitude areas are night blooming and have a wide range of timing with regard and pollen germination. Anther dehiscence one day before anthesis, which in turn confirmed the protandrous condition of the flower. Sreekala et al. (2007 2008) reported similar observations in *Impatiens henslowiana* Arn. and *Impatiens coelotropis* Fischer. Study of anthesis and anther dehiscence is vital for subsequent dispersal of pollen grains into the atmosphere (Bhattacharya and Mandal 2000).

It has been observed that each flower produced 5 stamens and an ovary with 5 cells. The mean number of pollen grains and ovules per flower are 38,280 and 20 respectively. Hence the pollen ovule ratio had been worked out as 1914 pollens per ovule (1974:1). In *Impatiens trichocarpa*, pollen grains are spherical and average of 32.72 µm in diameter (Table 1). The acetocarmine glycerin staining techniques revealed that 88% pollen grains were fertile. Pollen viability by FCR (fluorochromatic reaction) test confirmed that 82% pollen grains were viable on the day of anthesis (Figure 1e). Pollen viability gradually decreased on successive

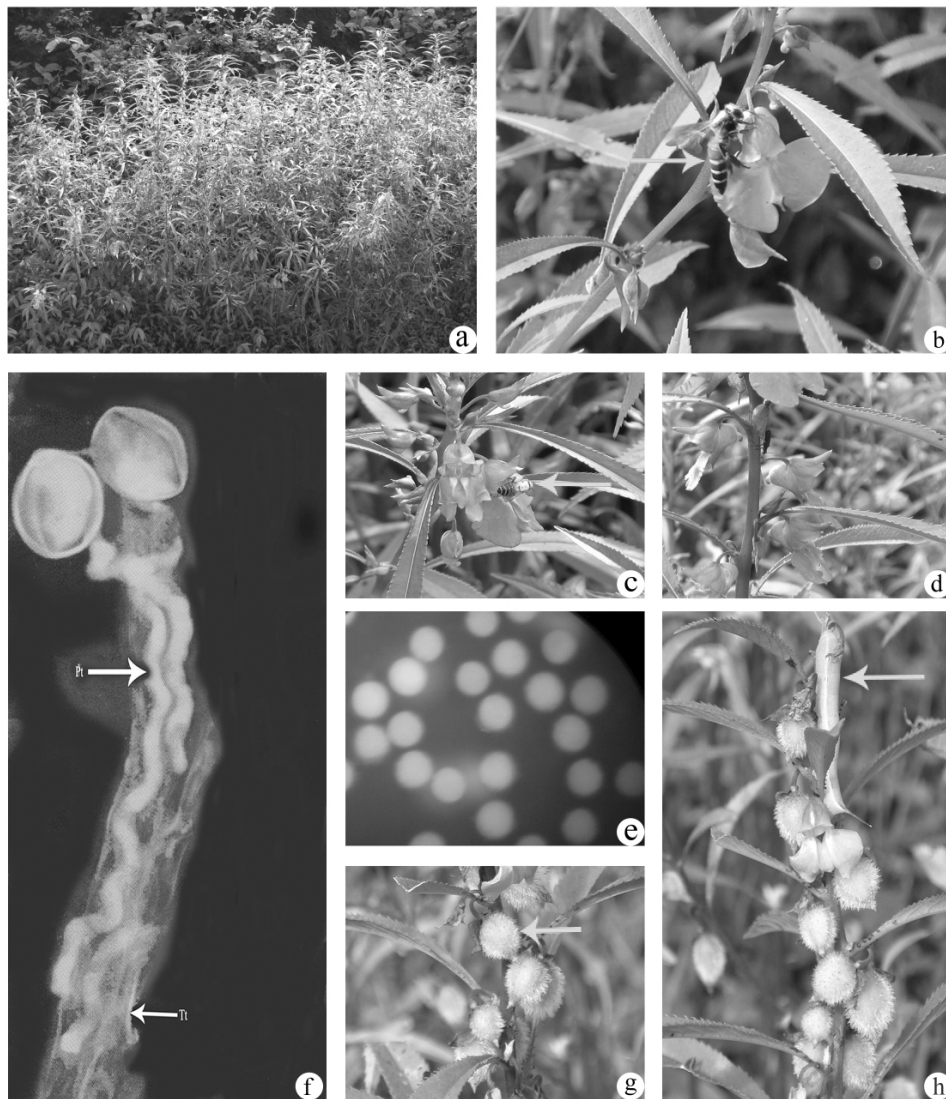


Figure 1. a. A population of *Impatiens trichocarpa* Hook. f.; b-d. Flower visitors : b- *Apis dorsata*, c- *Apis indica* and d- beetle, e. Pollen viability; f. *In vivo* pollen germination, g. Fruiting branch (fruits developed through xenogamy); h. Caterpillar infestation. Pt = Pollen tube, Tt = Transmitting tissue.

Table 2 Pollinators and their behaviour in *Impatiens trichocarpa*

Visitor species	Foraging nature	Stigma touch	Foraging hours
<i>Apis florea</i>	Nectar and pollen	++	0700-1000h
<i>Apis indica</i>	Nectar and pollen	++	0730-0930h
<i>Apis dorsata</i>	Nectar and pollen	++	0500-0900h
<i>Macroglossum variegatum</i>	Nectar	+	0300-0530h
<i>Pachliopta aristolochiae</i>	Nectar	+	0700-1600h
<i>Euploea core</i>	Nectar	+	0800-1700h
<i>Papilio demoleus</i>	Nectar	+	0830-1330h
<i>Chrysomya megacephala</i>	Nectar	+	0800-1200h

++- Good, +- Moderate

Table 3. Fruit set in *Impatiens trichocarpa* from 50 flowers pollinated in different modes

Pollination mode	Total No. of Fruits	% Fruits Set	Average seed weight, mg
Natural pollination (T)	29	58	6.5
Autogamous			
Self-pollination (T1)	0	0	0
Geitonogamy (T2)	32	64	7.0
Xenogamy (T3)	36	72	7.4

days after anthesis. This observation is similar to that of *Impatiens reptans* Moon in China (Tian et al. 2004), in which pollen viability was highest on the day of anthesis and thereafter decreased. Successful seed sets and establishing newer population generally depends up on viable pollen grains. *In vitro* pollen germination study revealed that 74% germinating pollen along with 680 μ m tube development was noticed in 5% sucrose medium. In the present investigation, the best pollen germination (76%) along with 864 μ m tube length was achieved in Brewbakers medium after 6 hr of incubation. Sugar acts as a nutritive material for pollen germination and it helps in maintaining proper osmotic balance between the germination media and pollen cytoplasm (Johri and Vasil 1961). Besides the medium contains carbohydrates, boron and calcium are other important substances required for pollen germination and tube elongation (Brewbaker and Kwack 1963).

In *Impatiens trichocarpa*, the flowers are beautifully coloured and start opening in the night and remain fresh till the morning. The nectar secretion begins one hour after anthesis and continued up to 24 hr after flower opening. Nectar secretion gradually decreased on successive days after anthesis. The nectar is present in the lip and spur of the flower and is lined with nectar secreting cells. The concentration of nectar in *Impatiens trichocarpa* ranged from 22 to 28%. The average volume of the nectar available per flower was $4.8 \pm 0.72 \mu\text{L}$. Pollination is effected by honeybees (*Apis indica* Fabricius, *Apis dorsata* Fabricius and *Apis florea* Fabricius), Hawk Moths (*Macroglossum variegatum* Rothschild & Jordan), Butterflies (*Pachliopta aristolochae* Fabricius, *Euplea core* Cramer and *Papilio demoleus* Linn.) and flies (*Chrysomya megacephala* Fabricius). They are visiting the flowers between 0300 and 1700h for nectar and pollen

(Table 2). Of the total visits, honey bees made 62% hawk moths 10%, butterflies 22%, and flies 6%. Among the honey bees *Apis indica* and *Apis florea* forage during 0700-100h, whereas the other species (*Apis dorsata*) during 0500-0900h (Figure 1b-c). Honeybees forage for both nectar and pollen. Observations on different body parts indicate that *Apis dorsata* have a higher amount of pollen grains on their body parts than other sps. Hawk moths forage in the night time between 0300-0530h. Butterflies and flies forage during day time between 0700-1700h. There is a strong relationship between weather and foraging activities of pollinators. When the weather is fine butterflies are more active and spend an average of 2-8 seconds per flower. However, during the rainy season butterflies were completely inactive. It was noticed that beetles are also rarely visited the flowers during the peak period of flowering (Figure 1d)

It is well known that the flowers of *Impatiens* have enormous diversity and different pollinators. Our studies show that *Impatiens trichocarpa* is pollinated by honey bees, hawk moths and butterflies, similar to those pollinating *Impatiens dalziellii* which has a more restricted distribution (Kulloli and Sreekala 2009). In subtropical regions of Africa, *Impatiens* species are pollinated by humming birds as well as by insects (Grey-Wilson 1980) whereas in temperate zones, the pollinators are bumble-bees and humming birds only (Rust 1977, 1979; Heinrich 1979 and Kato et al. 1989). Hence, in different climatic regions, species of pollinators vary. In Sumatra, one species of *Impatiens* such as *Impatiens platypetala* Lindl. was pollinated by crepuscular hawk moth such as *Macroglossum corythus* Walker (Kato et al. 1991). Some hawk moths (*Macroglossum* spp.) also visited *Impatiens* flowers in Japan (Kato et al. 1988). In the present study, a species of hawk moth (*Macroglossum variegatum*) visited the flowers of *Impatiens trichocarpa* Hook. f. during night time. They help the flower in pollination by their rapid and frequent visit to the flowers for nectar collection. Among honeybees *Apis dorsata* is the most important pollinator and visits more flowers than any other pollinators during day time for nectar collection. This observation was similar to that of *Impatiens coelotropis* Fischer (Sreekala et al. 2008). All insects are pollen carriers and their frequent interplant movement facilitates cross pollination. About 15% of flower buds and 8% of open flowers were infested with unidentified larvae and caterpillar (Figure 1h). The larvae cut the basal parts of the flowers (spur) containing nectar. The infested flowers were not visited by the pollinators.

Thus in all 23% of the buds and open flowers were damaged to the point of producing no fruits.

Flowers are cross-pollinated as confirmed by various hand-pollination experiments. In natural cross pollinations, 58% fruit set was observed. Fruit set was not observed in autogamous self pollination. However, 64% fruit set was observed in geitonogamy and 72% in xenogamy respectively (Table 3, Figure 1g). Capsules developed through geitonogamy and xenogamy had more seeds than capsules produced by natural pollination. The average weights of individual seeds produced by the different treatments (T, T2 and T3) were 6.5, 7.0 and 7.4mg respectively, which indicated that robust and healthy seeds were produced by artificial pollination than natural system. The fruit took 15-17 days for attaining maturity. Each capsule contains an average of 14.2 ± 2.34 seeds. The seeds obtained through natural and artificial pollination were germinated up to 30% only.

Pollination studies can provide a lot of information about the loss of many species, because pollination is the fundamental step in plant reproduction. Monica and Bhatnagar (2007) reported that the decline of many entomophilous plants may be due to unsuccessful pollination because of loss of their pollinators. For out-crossing of entomophilous plants, population size and plant density are closely associated with the attraction and activity of pollinators. Obligate outbreeding is the predominant reproductive strategy in tropical ecosystems, either through self incompatibility mechanisms or various forms of functional dioecy. In the present study, different hand pollination experiments demonstrated that *Impatiens trichocarpa* is a self incompatible, obligate outcrosser. Manipulated geitonogamy and xenogamy has given 64-72% fruit set whereas in open pollination it is 58%.

Stigma receptivity is a critical factor for successful completion of post pollination events. Usually it is maximum soon after anthesis but it varies from species to species (Shivanna and Johri 1989) but in the candidate species maximum stigma receptivity (100%) was observed after 8 hrs of anthesis by showing 56% *in vivo* pollen germination along with $664 \pm 0.942 \mu\text{m}$ long pollen tube on the stigmatic surface (Figure 1f). Subsequently, the stigma receptivity and *in vivo* pollen germination were decreased on successive days after anthesis which considerably affect the fruit and seed production.

CONCLUSION

Impatiens trichocarpa Hook. f. reproduce by means of cross pollination. In natural conditions 58% fruit set was observed whereas the artificial cross pollination enhanced up to 72%. However the seed germination was limited to 30%, which may be one of the major reason for poor seedling recruitment. The species is poorly distributed in the wild mainly because of its adaptations for high altitude, fragmentation of populations, narrow environmental niche, poor seed germination, infestation by insects etc. All these causal factors either alone or in combination with others are responsible for limited distributions in natural condition.

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