# Reproductive ecology of *Impatiens platyadena* Fischer, a critically endangered balsam of Western Ghats

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***Impatiens platyadena* is a critically endangered balsam occurring in isolated pockets of the Western Ghats of Kerala. In order to understand its narrow distribution and endangerment, we studied reproductive ecology covering floral phenology, pollination, pollen–pistil interactions and breeding systems. The creamy-red-coloured flowers bloom in the early morning between 0600 and 0830 h, but the anther dehisces one day before anthesis. The pollen–ovule ratio (2346 : 1) revealed that the species is adapted to entomophilous pollination. Pollen viability and stigma receptivity are temporally isolated; stigma is receptive on the third day and pollen viability is highest (75%) on the day of anthesis. Field observations confirmed that the flowers offer both pollen and nectar to the visitors. The visitors include honey bees and butterflies, but the former served as better pollinators. Manual pollinations revealed that the species permits both geitonogamous and xenogamous pollination. The fruit set rate in natural pollination is low (38%), but manual xenogamous pollination enhanced the fruit set up to 65%. Its dependence on specialized habitats, fragmentation of populations, narrow environmental niche, scarcity of pollinators, delayed stigma receptivity and low percentage of seed germination could be the reasons for its limited distribution and endangerment in the Western Ghats.**

**Keywords:** Critically endangered, *Impatiens platyadena*, narrow distribution, reproductive ecology.

ANY conservation approach has to be based on an in-depth study and understanding of plant and its environment, including reproductive biology which determines the fitness of the species in a given community. Adequate knowledge on reproductive biology is essential for the conservation, management and recovery of endemic and endangered species. By studying the reproductive biology of rare, endangered and threatened (RET) species, one can understand the exact causal factors inducing rarity and can overcome these factors through scientific intervention, so as to protect the plants from endangerment.

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Balsams are beautiful plants bearing curious and variously coloured flowers with peculiar floral structures. The members are more or less succulent, annual or perennial herbs, rarely becoming shrubs or epiphytes. Majority of the wild balsams have great horticultural potential due to their diversified forms, shapes and spectacular colours (A. G. Pandurangan, unpublished). Balsams belong to the family Balsaminaceae, consisting of about 1000 species with two genera, viz. *Hydrocera* Blume and *Impatiens* L.[1]. They are mainly distributed in the tropics and subtropics of the old world, but a few species occur in the temperate Eurasia and North America. In India, the genus *Impatiens* is represented by 209 species[2] and is mainly distributed in three major centres of diversity, i.e. Western Himalayas, Hills of the North Eastern States and the Western Ghats. Ninety-two balsam species have so far been recorded from Peninsular India, of which more than 80 are endemic and confined to the Western Ghats, with 30 species already in the threatened category, including 19 critically endangered ones[3] (A. G. Pandurangan, unpublished). Though ideal climatic conditions prevailing in the Western Ghats region provide suitable habitat for balsams, their populations are rapidly declining due to various biotic and abiotic factors[4,5]. Reproductive inefficiency may also lead to the rarity of balsams in certain cases. *Impatiens platyadena* was described by Fischer[6] based on the collection of Barnes. During our present study, *I. platyadena* was collected from isolated localities in and around Pettumudi, Rajamalai and Neymakkad gap of Idukki District, Kerala, India. Its type locality is possibly surviving in a few fragmented populations scattered in a narrow range. Being subjected to the pressures of habitat destruction, the few remaining populations of this species are on the verge of extinction. This species has been recorded as critically endangered by Nair[7]. There is extreme fluctuation in the population and number of individuals due to anthropogenic pressures in the shola forest ecosystem[4]. However, comprehensive studies on reproductive ecology of endangered balsams are scarce, due to their high-altitude habitat, delicate structure and explosive fruits. We studied the reproductive ecology of one such critically endangered balsam, viz. *I. platyadena* to find out the possible reasons for its limited distribution and endangerment.

The study area comprises mainly the high-altitude regions (Neymakkad gap, Rajamalai and Pettumudi Idukki District) of the Western Ghats (Figure 1), with altitude ranging from 1600 to 2400 m asl. Based on the study, it was estimated that the extent of occurrence and the area of occupancy of the species were less than 10 and 1 sq. km respectively. The mature individuals were calculated as ±100 in the entire distributional areas. Three populations of 25 plants each were selected in the natural habitat for observations on phenology, pollination and breeding systems. Flowering phenology was observed on a day-to-day basis, which includes flowering season,

flower development, anthesis, anther dehiscence, etc. according to the method of Dafni *et al.*[8]. Morphology of the flower and flower parts was studied using hand lens and dissecting microscope. Mature and undehisced anthers were collected and the total number of pollen grains per anther was calculated. Pollen–ovule ratio was calculated according to the method of Cruden[9]. Pollen viability was assessed by fluorochromatic reaction test (FCR), 2,3,5-triphenyltetrazolium chloride (TTC) test[10] and diaminobenzidine (DAB) test[8]. To study the pollen germination *in vitro*, pollen grains collected from the fresh flowers were incubated in Brewbakers medium at different time intervals. Pistils of 24 h before anthesis to the third day of anthesis were used to study stigma receptivity. Stigma receptivity was assessed using hydrogen peroxide[11] and surface stigma esterases analysis[10]. *In vivo* pollen germination was studied by aniline blue fluorescent microscopic method. Scanning electron microscopic studies were made to study the surface features of stigma and *in vivo* pollen germination.

Observations were made on floral visitors regularly during the flowering season. The number of floral visitors, percentage of visits made by insects and stigma touch were calculated. The foraging behaviours of visitors were analysed by photography and visual observations. For each population, an observation was made to assess the number of floral visitors visited per hour per population. The pollen carrying capacity of floral visitors was analysed and the pollinators confirmed according to the method suggested by Herrera[12]. After each visit of the pollinators, the stigmas were observed by hand lens and the transfer of pollens confirmed by their visit to the virgin stigma. Insects were trapped using butter-paper bags while they were foraging the flowers and were fixed in ethyl acetate vapours for identification. The foraging period and the type of food collected by different pollinators/visitors were also recorded by close observation.


**Figure 1.** Study area – distributional areas of *Impatiens platyadena* in the southern Western Ghats.

Different breeding experiments such as manual self-pollination (autogamy), geitonogamy, xenogamy and open pollination were conducted in the field. The pollination experiments were carried out on selected plants at the time of maximum stigma receptivity. In order to observe the percentage of fruit set, 100 flowers were tagged for each breeding experiment, including open pollination. The number of fruits developed was recorded with respect to the number of flowers. The following xenogamous pollination experiments were also conducted among three populations to check the out-crossing level, i.e. ($T_1$) – populations of Neymakkad × populations of Pettumudi; ($T_2$) – populations of Pettumudi × populations of Rajamalai and ($T_3$) – populations of Rajamalai × populations of Neymakkad.

Fruit initiation, development, maturation and dispersal of seeds from the mother plant were carefully observed. The seeds were collected and preserved for further studies. To analyse the moisture content of the seeds, the mature capsules were collected randomly before dehiscence. Seed moisture content was calculated using the formula developed by the International Seed Testing Association[13]. The viability of the seeds was analysed according to the method suggested by Enescu[14]. The seeds were allowed to germinate in the natural habitat ($1 \times 1$ m plots). They were also collected and stored under laboratory conditions and allowed to germinate in plastic trays filled with garden soil.

*I. platyadena* is an erect herb (0.5–1 m) growing in the deep shola forests. The inflorescence is an axillary raceme in peduncles with slightly drooping habit. The flowers are bright red with pale pinkish spur and scarlet petals (Figure 2 *a*). The capsules are small, fusiform with pyriform brown seeds. In *I. platyadena*, the new seedlings appeared in the first week of June, with the onset of monsoon. Flowering starts in September and extends up to December, with a peak during November. About 62% of *Impatiens* species prevailing in the Western Ghats flower during July–December, 16% during April–June and 15% during January–March. Interestingly, 18% flower throughout the year if conditions are favourable[15]. The flowering period extends up to 120 days in a year and

average lifespan of each flower is 3–4 days. Each plant produces 3–5 inflorescences and each inflorescence produces 6–9 flowers during the peak flowering season. Fruit takes 12–15 days for attaining maturation after pollination. Fruit maturation and dehiscence start in the first week of October.

Generally, majority of the flowers of wild balsams growing in the high-altitude areas of the Western Ghats are night-blooming and have a wide range of timings with regard to pollen germination[16]. But in *I. platyadena*, anthesis commences in the morning between 0600 and 0930 h and anther dehisces one day before anthesis. The mean number of pollen grains and ovules per flower is $37{,}530 \pm 24.06$ and $16 \pm 2.13$ respectively. Hence, the pollen–ovule ratio had been worked out as 2346 : 1. Successful seed set and establishing newer populations generally depend upon viable pollen grains. Pollen viability by FCR test confirmed that 75% of the pollen grains were viable on the day of anthesis (Figure 2 *b*). Pollen viability by TTC tests and DAB test showed that 74% and 73% of pollen grains respectively, were viable on the day of anthesis, and gradually lose their viability on successive days after anthesis. Only 9% of the pollen grains were viable on the third day of anthesis. *In vitro* pollen germination studies showed that there was no pollen germination at the time of anther dehiscence, i.e. one day before anthesis. The best pollen germination (76%) along with $796 \pm 0.235$ µm tube length was noticed in Brewbakers medium on the day of anthesis. However, there was substantial reduction in the percentage of pollen germination on the second (26%) and third (8%) day of anthesis.

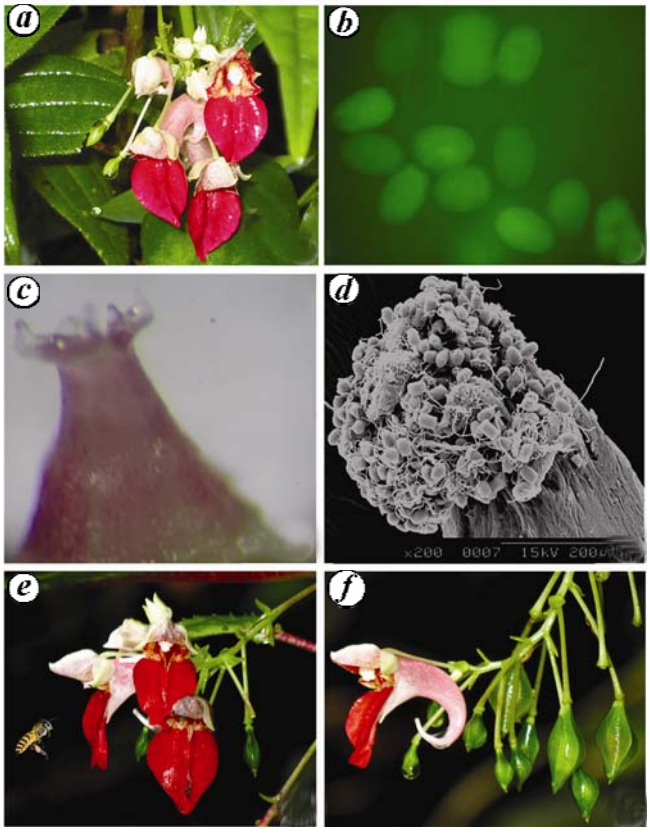


**Figure 2.** ***a***, Flowering branch of *I. platyadena*. ***b***, Pollen viability tested by FCR test. ***c***, Receptive stigmatic surface of *I. platyadena*. ***d***, SEM photomicrograph of *in vivo* pollen germination. ***e***, *Apis cerana* foraging the flower and ***f***, Fruit set at xenogamous pollination experiment.

In *I. platyadena*, the dithecal tetra-sporangiate anthers are connivent and open either apically or laterally by means of pores or slits. They lie as a cap above the gynoecium with five stigmas. There is no chance for pollen grains come into contact with their own stigmatic surface. Only after the pollen has been released and the androecium shed will the star-shaped stigmas ripen. The coherent stigma commonly spread and the star-shaped receptive surface is exposed (Figure 2 *c*). The stigma is wet and non-papillate type. Irrespective of the morphology, the stigma invariably contains extracellular components on the receptive surface. Stigma esterases were not observed one day before and up to two days after anthesis. Non-specific surface stigma esterases were observed throughout the stigma mainly on the stigmatic lobes and slightly on the stigmatic head on the third day of anthesis. The present study showed that intense/copious presence of esterases on stigmas during their high receptive period might be considered as one of the associated factors of stigma receptivity. The presence of esterases become more in stigmas after anthesis, which often coincides with more receptivity[17,18]. Maximum percentage of *in vivo* pollen germination (65) along with 1026 µm long pollen tube was observed on the third day of anthesis (Figure 2 *d*), but at the same time the pollen viability reduced drastically to 9%.

The major visitors of *I. platyadena* are honey bees (*Apis cerana*, *Apis dorsata*, *Trigona iridipennis*) and butterflies. They are attracted by the bright colour and mild fragrance of the flowers. Nectar is present in the lip region where the inner surface is lined with nectar-secreting cells. The average volume of nectar available per flower on the day of anthesis and the third day of anthesis was $8.3 \pm 1.82$ µl and $4.2 \pm 1.61$ µl respectively. The concentration of sugar in the nectar ranged from 20% to 24%. Nectar quantity was gradually reduced on successive days after anthesis. Honey bees forage both nectar and pollen. They land in front of the flower and pass through the corolla to the spur behind the sepal to collect nectar (Figure 2 *e*). While collecting nectar from the spur, their back comes in contact with the androecium. When the insect comes in contact a second time, the androecium falls off and the ovary with stigma becomes exposed. The arms of the stigma then open gradually and become receptive (Figure 2 *c*), so that when the next insect visits the flower for nectar, its back comes into contact with the stigma and cross-pollination takes place. Finally, the perianth

**Table 1.** Pollinators and their foraging behaviours in *Impatiens platyadena*

| Visitor | Visiting time | Foraging nature | Foraging hours | MNIV | Percentage of visit | Stigma touch |
|---|---|---|---|---|---|---|
| *Apis cerana indica* | Day | Nectar and pollen | 0730–1600 | 12.34 | 21.07 | +++ |
| *Apis dorsata* | Day | Nectar and Pollen | 0900–1300 | 11.78 | 20.11 | +++ |
| *Trigona iridipennis* | Day | Nectar | 0630–1000 | 09.52 | 16.25 | ++ |
| *Danaus chrysippus* | Day | Nectar | 0600–1300 | 04.21 | 07.18 | + |
| *Danaus genutia* | Day | Nectar | 0700–0900 | 07.03 | 12.00 | ++ |
| *Tirumala limniace* | Day | Nectar | 0600–1100 | 05.24 | 08.95 | + |
| *Parantica aglea* | Day | Nectar | 0700–1600 | 02.43 | 04.15 | + |
| *Caprona ransonnetti* | Day | Nectar | 0800–1200 | 06.03 | 10.29 | ++ |

MNIV, Mean number of individuals visited/h/population; +++, Very good; ++, Good, and +, Poor.

**Table 2.** Fruit set in different modes of pollination in *I. platyadena*

| Breeding experiment | No. of flowers pollinated/observed | No. of flowers setting fruits | Mean no. of seeds/capsule | Percentage of seed set |
|---|---|---|---|---|
| Open pollination | 100 | 38 | 8.39 | 52.4 |
| Autogamous self-pollination | 100 | 0 | 0 | 0 |
| Geitonogamous pollination | 100 | 41 | 8.88 | 55.5 |
| Xenogamous pollinations | | | | |
| Within the populations | 100 | 48 | 8.67 | 54.2 |
| Between the populations | | | | |
| $T_1$ (Neymakkad × Pettumudi) | 100 | 65 | 9.12 | 57.0 |
| $T_2$ (Pettumudi × Rajamalai) | 100 | 43 | 8.93 | 55.8 |
| $T_3$ (Rajamalai × Neymakkad) | 100 | 48 | 9.0 | 56.3 |

falls off and the insect visits cease. This observation was supported by the findings of several authors in *I. pallida*, *I. capensis*[19] and *I. reptans*[20]. *T. iridipennis* forage during 0630–1400 h for pollen and loads were found on the hind limbs and heads (Table 1) Butterflies (*Danaus chrysippus*, *Danaus genutia*, *Tirumala limniace* (Blue tiger), *Parantica aglea* (Glassy tiger) and *Caprona ransonnetti* (Golden angle)) visited the flowers, spending 4–8 s/flower. Butterflies land on the lower two wing petals, slightly bend their body and insert the proboscis in the long and deep succate lip region to collect nectar from the flower. During the nectar harvesting time, pollen grains stick on the head of the butterflies and are transferred to the stigma. There was a relationship between the butterfly activities and weather condition. When the weather was bright, butterflies actively visited the flowers of *I. platyadena*, but when it was cloudy they were less active. On rainy days, they were completely inactive. Similar results were observed by several authors[19,20], who confirmed that honey bees and butterflies are the major pollinators of *Impatiens*.

To ascertain whether pollination is a constraint or not for low seed set under open pollination, 100 stigmas from different populations were examined under the microscope on the third day. About 50–80 pollen grains were observed in 20% of the stigmas; 30–50 in 33% of the stigmas, and 47% of the stigmas contained below 30 pollen grains. As there are 16 ovules per ovary, the number of pollen grains received on the stigmas appeared to be sufficient to effect pollination, fertilization, and fruit and seed set.

In *Impatiens*, the morphology, size and shape of the flowers may vary between the species, but the breeding system of the species is almost the same[21]. Hundred flowers each were selected from different populations for breeding experiments. In natural condition, 38% fruit set was observed. Fruit set was not observed in autogamous self-pollination. In manual geitonogamous and xenogamous pollination experiments, the percentage of fruit set was markedly higher than that of open pollination (Table 2). Highest percentage of fruit set (65) was observed in cross-pollination (Figure 2 *f*) between Neymakkad × Pettumudi ($T_1$). The fruit and seed setting percentage was comparatively increased by manual xenogamous pollination experiments in other endangered *Impatiens*[20,21].

Fruit dehiscence and seed dispersal are a unique phenomenon in *Impatiens*. Seed dispersal is a critical event in the life cycle of plants and influences the genetic structure of populations. Undehisced mature capsules were covered with paper bags and the seeds were collected. The moisture content of the seeds was regularly assessed, which revealed that there was a sudden reduction in the moisture content from 69% to 29% within a month. The viability of the seeds at the time of dehiscence was 44%

and extended its viability up to 12 months. The percentage of seed germination was only 30 in both natural habitat and laboratory conditions. Freshly harvested seeds show no germination. The seeds exhibit dormancy period to overcome the dry seasons of February–July. After 6 months, the seeds start germinating, but at the same time viability of the seeds reduces to 21%. The germination capacity was also gradually reduced after 10 months and there was no seed germination after 12 months. The new seedlings emerged in the first week of August. The present study supports the previous works[4,22,23] in which the seeds of several high-altitudes *Impatiens* exhibit dormancy period to overcome the dry months of February–July. In addition, the seedlings were heavily affected by fungal disease and browsing of herbivores. Because of the above reasons, less than 5% of the seedlings were recruited to the populations. Poor seedling recruitment was also observed in *I. coelotropis*[22] due to the damping-off disease, and infestation by other insects and pests.

In the present study, the percentage of fruit set in open pollination of *I. platyadena* was low, but cross-pollination, especially through xenogamous pollination enhanced the fruit set. In this case, the extent of occurrence of *I. platyadena* is restricted to less than 10 sq. km; hence the availability of disjunct, viable population is a limiting factor for gene flow which greatly affects the sexual reproduction. This species is poorly distributed in the wild mainly because of its adaptations to high altitude, fragmentation of populations, narrow environmental niche, delayed stigma receptivity, scarcity of pollinators, and low percentage of seed germination.


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