RESEARCH COMMUNICATIONS


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Reproductive biology of Crataeva religiosa Forst.

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Crataeva religiosa flowers profusely during March to May, when it sheds all its leaves. The flowers are large, hermaphrodite, actinomorphic and complete. Flowers open in the evening between 1900 and 2030 h followed by another dehiscence at 1930–2100 h. Flowers offer pollen and nectar to the visitors, which include honey bees, moths, butterflies, bugs and birds. The plant is self-incompatible and obligate out-crosser. Fruit-set is restricted to only 22%. The beauty of the flowers as well as fruit production are adversely affected by the formation of floral galls induced by the insect, Neolasiop tera crataevae Mani, order Diptera.

Keywords: Crataeva religiosa, floral galls, Neolasiop tera crataevae, reproductive biology.

CRATAEVA RELIGIOSA Forst. (family Capparidaceae) is a large tree distributed in the tropical zone and is common throughout India, Myanmar and Sri Lanka, either wild or cultivated1. It is cultivated in the gardens for its ornamental as well as medicinal value2. Despite its usefulness, less

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attention has been paid to its floral biology, breeding systems and pollination mechanism. These are essential for the conservation, improvement and establishment of cultivation to increase the frequency of occurrence of this species. Keeping these facts in view, a detailed study on the reproductive biology of C. religiosa plants growing in different parts of Agra city has been made.

Flowering phenology was observed at plant and inflorescence level with reference to day-to-day flowering pattern in twenty-five marked plants. For the latter, 200 inflorescences, selected at random from different individuals were tagged before the initiation of flowering. These individuals were followed daily and the number of open flowers was recorded. The open inflorescences were then removed to avoid recounting the next day. The tagged inflorescences were followed until they ceased flowering. One hundred flowers were sampled to record the floral morphology and pollen characters. Anthesis, anther dehiscence and stigma receptivity were studied using various methods as described by Shivanna and Rangaswamy \(^3\). The number of pollen grains/anther/flower was determined from 25 flowers following Cruden \(^4\). Pollen size was measured with an ocular micrometer under light microscope following the procedure of McKone and Webb \(^5\). The number of pollen grains divided by the number of ovules per flower will yield the pollen–ovule ratio \(^6\). Pollen viability was assessed by hanging drop method after Brewbaker and Kwack \(^7\). In vivo pollen germination was checked by aniline blue fluorescence microscopic method as described by Shivanna and Rangaswamy \(^3\). Breeding behaviour by autogamy, geitonogamy and xenogamy was tested using controlled pollination studies. In order to observe the rate of natural fruit-set, two hundred inflorescences on different trees were tagged and were followed until fruit development. Foraging behaviour of insects and birds was recorded. They were observed with binoculars and photographed. Foraging activity during night was observed using a torchlight. Pollination efficiency of different bees was checked by observing the pollen load on different body parts under a microscope, according to the procedure given by Kearns and Inouye \(^8\).

*C. religiosa* sheds all its leaves before the initiation of flowering (Figure 1a). Flowering starts from 26 March and continues till the last week of May, with maximum bloom in April. A limited number of flowers continues to appear even during June and July. A flush of new trifoliate leaves appears with the cessation of flowering by the end of May. The inflorescence is a corymbose–raceme and consists of 25 ± 3.0 flowers (R 19–30; Table 1, Figure 1b). Flowers are large, hermaphrodite, actinomorphic, hypogynous and complete (Table 1, Figure 1c). The calyx is gamosepalous with four sepals, and corolla is polypetalous with the same number of petals that are arranged alternate to sepalas (Table 1). Stamens are numerous, as many as 25 ± 1.5 (R 18–31) (Table 1). They are polyandrous, adnate to the base of the gynophore, longer than the petals, bicelled and introrse. Single pistil is differentiated into stigma and ovary. Stigma is capitate, dry and non-papillate (Table 1). The ovary is bicarpellary, syncarpous (Table 1) and elevated on a long stalk called gynophore. The number of ovules/ovary is 90 ± 9.7 (R 76–106), lying on two parietal placentae (Table 1).

The flowers open between 1900 and 2030 h (Table 1). Opening of the flowers is confined to the hours of darkness. Nectary is present at the base of the gynophore. Anthers dehisce by longitudinal slit around 1930–2100 h. Pollen grains are spherical, tricolpate with reticulate exine and 23.5 µm in diameter. The mean number of pollen grains per anther is 3510 and per flower is 87,750. The ratio of pollen grains to ovule number is 975 : 1. The stigma becomes receptive after anther dehiscence around 2200 h and remains so until the late morning (0800–0930 h) of the next day. In *vitro* pollen germination studies indicate that the pollen grains remain viable for 16 h after anther dehiscence. At the time of anther dehiscence, 94% of the pollen grains are viable. However, there is substantial reduction in pollen viability after 10 h (50%) up to 16 h (3%) (Table 2). In *vivo* pollen germination studies show 58% pollen germination after 10 h (Figure 1f) and germination percentage is only 7% after 16 h on the stigmatic surface (Table 2).

Each flower lasts for two days. At initial bud stage petals are green in colour, but acquire white colour at late bud

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**Table 1.** Floral characters of *Crataeva religiosa*

<table>
<thead>
<tr>
<th>Floral character</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflorescence</td>
<td>Corymbose-raceme</td>
</tr>
<tr>
<td>No. of flowers/inflorescence</td>
<td>25 ± 3.0</td>
</tr>
<tr>
<td>Flower</td>
<td>Hermaphrodite, actinomorphic,</td>
</tr>
<tr>
<td></td>
<td>complete</td>
</tr>
<tr>
<td>No. of stamens</td>
<td>25 ± 1.5</td>
</tr>
<tr>
<td>Calyx</td>
<td>Gamosepalous with four sepals</td>
</tr>
<tr>
<td>Corolla</td>
<td>Polypetalous with four petals</td>
</tr>
<tr>
<td>Time of flower opening</td>
<td>1900–2030 h</td>
</tr>
<tr>
<td>Time of anther dehiscence</td>
<td>1930–2100 h</td>
</tr>
<tr>
<td>Mode of anther dehiscence</td>
<td>Longitudinal slit</td>
</tr>
<tr>
<td>Pollen grains/anther</td>
<td>3510</td>
</tr>
<tr>
<td>Pollen grains/flower</td>
<td>87,750</td>
</tr>
<tr>
<td>Pollen size</td>
<td>23.5 µm</td>
</tr>
<tr>
<td>Stigma type</td>
<td>Capitate, dry and non-papillate</td>
</tr>
<tr>
<td>Ovary type</td>
<td>Bicarpellary and syncarpous</td>
</tr>
<tr>
<td>No. of ovules/ovary</td>
<td>90 ± 9.7</td>
</tr>
</tbody>
</table>

**Table 2.** Pollen germination at different time intervals after anther dehiscence

<table>
<thead>
<tr>
<th>Hours after anther dehiscence</th>
<th>In <em>vitro</em> pollen germination (%)</th>
<th>In <em>vivo</em> pollen germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>50 ± 0.5</td>
<td>58 ± 0.3</td>
</tr>
<tr>
<td>12</td>
<td>30 ± 1.4</td>
<td>35 ± 1.2</td>
</tr>
<tr>
<td>14</td>
<td>12 ± 2.62</td>
<td>18 ± 2.50</td>
</tr>
<tr>
<td>16</td>
<td>3 ± 0.92</td>
<td>7 ± 0.76</td>
</tr>
</tbody>
</table>

±, Standard deviation.
Figure 1. Crataeva religiosa. a. Tree in flowering; b. Flowering branch; c. Normal flower; d. Partially infested flower; e. Fully infested flower; f. In vivo pollen germination on stigmatic surface showing pollen tubes (pt); g. Apis dorsata; h. Psittacula krameri.

stages. At the time of anthesis petals remain white, but in the morning of the next day they gradually turn light yellow and finally become dark yellow by the evening. The next morning, the petals begin to wither and by the evening petals and anthers from pollinated flowers drop-off, leaving only the sepals and pistil. Change in floral colour in Helicteres isora is well known, as the colour changes from bluish-grey to dark red.

The beauty of flowers as well as fruit production is adversely affected by the formation of floral galls induced by...
the insect, Neolasioptera crataeaeva, Mani, order Diptera. Galls start developing a few days after the initiation of flowering. Their number increases with increase in floral density and then declines with rise in temperature. Percentage of gall formation is low in young plants, but higher in older plants. Galls usually develop from meristematic and young tissues of the thalamus and grow towards the base of the anther filament and gynophore, which become bulbous. Infestation of the insect on a single flower is usually not uniform as is evident by the presence of normal, semi-infested and fully infested floral parts in the same flower. Quite often, the whole flower gives rise to an irregular mass somewhat depressed above and funnel-shaped at the base. During gall formation, floral components are highly modified. Those near the galls are severely affected, whereas the ones away from the zone of infection tend to be normal. Sokhi and Kapil have also reported morphological changes in the flowers of Terminalia arjuna induced by the insect Trioza.

Fruiting starts in the second week of April and fruits begin to mature in the last week of May. Mature fruits start to disperse their seeds during the second week of June. Natural fruit-set is 22%. A sample of 200 inflorescences consisting of 5024 flowers, selected at random on different trees at flower stages was used for estimating fruit and seed-set rate. An inflorescence consists of 25 ± 0.5 flowers; among them 18 ± 1.2 are normal and 7 ± 0.5 are infested. Thus, among 5024 flowers, 3641 are normal and 1383 are infested. Among normal flowers, 897 flowers with 80,730 ovules set fruits with 50,456 seeds. Seed-set is 62%.

Among the infested flowers, 40% is partially infested (Figure 1d) and 60% fully infested (Figure 1e). None of these produces fruits. Thus, among all the flowers on a tree, 75% is normal and 25% infested. Among the 25%, 10% is partially infested and 15% fully infested. They are not visited by pollinators. Considering the total flower investment as 100%, the species loses 25% to infestation and another 53% to flower abortion, leaving just 22% for natural fruit-set.

Flowers are cross-pollinated as confirmed by various hand-pollination experiments. The fruit-set percentage through xenogamy is 92 and there is no fruit-set by other modes of pollination (Table 3).

The flowers are visited by honey bees (Apis dorsata; Figure 1g, A. indica and A. florea), moths (Achoria grisella), butterflies (Pieris brassicae and Danaus plexippus), wasps (Polistes hebraeus and Vespa sp.), bugs (Bagrada cruciferae) and birds, including sun birds (Nectarinia asiatica), parrot (Psittacula krameri; Figure 1h), koel (Eudynamys scolopaceus) and pigeon (Columba livia). They visit the flowers between 1900 and 0900 h for nectar and/or pollen. Of the total visits, honey bees made 61%, moths 15%, butterflies 8%, birds 7%, wasps 6% and bugs 3%. Among honey bees, A. dorsata forage during 1900–0900 h, whereas the other two species during 0500–0900 h. Honey bees forage for both nectar and pollen. Moths forage after anthesis. They are attracted by the white colour and heavy fragrance of the flowers. They forage during 1900–0100 h for pollen and nectar. Butterflies, wasps and bugs forage during 0500–0900 h for nectar only. All insects are pollen carriers and their frequent inter-plant movement facilitates cross-pollination. Birds forage during 0500–0900 h for nectar and for eating floral parts and fruits. Sun birds forage largely for nectar, whereas other birds visit the flowers for nectar and for eating floral parts and fruits. While collecting nectar, all these birds contact the stamens and stigma with their bill and forehead, both ventrally and dorsally and their frequent movements among the conspecific trees facilitate cross-pollination.

Observations of different body parts indicate that A. dorsata specimens have a higher amount of pollen grains on their body than other species (Table 4). It is the most dominant in number and visits the flowers soon after anthesis at night, showing brisk activity up to 0730 h. Later its activity declines and disappears at around 0900 h. This suggests that A. dorsata is capable of collecting forage during moon night hours. This bee is intolerant to excessively high temperatures and has thus developed adaptations to forage during night under moonlight. Rao and Raju have reported A. dorsata activity during night under moonlight on the flowers of Pterocarpus santalinus during March–May, to avoid excessively high temperature during daytime. The other honey bees too restrict their foraging schedules to the early morning time. Therefore, activities of pollinators are related to the ambient temperatures. Their activities are being limited by low temperatures in temperate climates and high temperatures in tropical climates.

Obligate outbreeding is the predominant reproductive strategy in tropical ecosystems, either through self-incompatibility mechanisms or various forms of functional dioecy.

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**Table 3.** Results of breeding systems in C. religiosa

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of flowers pollinated</th>
<th>No. of fruits</th>
<th>Fruit-set percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autogamy</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Geitonogamy</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Xenogamy</td>
<td>50</td>
<td>46</td>
<td>92</td>
</tr>
</tbody>
</table>

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**Table 4.** Pollen pick-up by bees on C. religiosa as revealed by observing pollen on different body parts

<table>
<thead>
<tr>
<th>Visitor species</th>
<th>Sample size</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apis dorsata</td>
<td>10</td>
<td>420–510</td>
<td>448 ± 345</td>
</tr>
<tr>
<td>A. indica</td>
<td>10</td>
<td>350–380</td>
<td>367 ± 310</td>
</tr>
<tr>
<td>A. florea</td>
<td>10</td>
<td>200–240</td>
<td>214 ± 186</td>
</tr>
</tbody>
</table>

±, Standard deviation.
Most of the Acacia species in the tropical ecosystems are found to be self-incompatible and out-crossing. Acacia simulata exhibits self-incompatibility, as indicated by Raju and Rao (12). Hand pollination experiments show that C. religiosa is a self-incompatible, obligate out-crossover. This breeding system is functional only when conspecifics bloom simultaneously and occur nearby, and requires vectors to mediate pollen among conspecific plants.

In C. religiosa, the natural fruit-set is low when compared to the high flower production. Different factors could affect fruit-set. First is the floral galls formation, which leads to loss of productivity. Secondly, the intensive pollen collecting behaviour of attending bees and their tendency to confine to the same plant that they first forage may result in more wasteful self-pollen transfer.

C. religiosa is of ornamental as well as medicinal importance and is an obligate out-crossover. For higher fruit-set, conspecific trees should be planted nearby and remedial measures should be adopted to combat infection of floral parts.


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Histological observations on the scleractinian coral Porites lutea affected by pink-line syndrome

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A pink-line syndrome (PLS) was reported in the reef building coral Porites lutea in Kavarattir island of the Lakshadweep archipelago. The affected corals had dead patches colonized by a cyanobacterium Phormidium valderianum and the bordering coral tissue was pink. We examined the histological changes associated with the PLS-affected tissue. Results showed that the zooxanthellae were released from the gastrodermal cells into the coelenteron. Gastrodermal cells undergo necrosis and detachment from the basal membrane. The basic staining of the cytoplasm in the gastrodermal cells bordering the calciloblastic layer suggests accumulation of calcium ions. The ectodermal epithelium and calcilobastic cells showed destruction through ‘apoptosis-like’ processes. Cell swelling and vacuolation were observed in the gastrodermal and ectodermal cells. In this communication we discuss how the presence of the cyanobacterium adjacent to the PLS-affected tissue could cause the observed damage, bring about imbalance and a shift in the coral-zooxanthellae symbiosis.

Keywords: Cellular changes, pink-line syndrome, Porites lutea, symbiosis, zooxanthellae.

EXISTENCE of corals is threatened by environmental changes, pollution and direct human interference in many coral reefs around the world. Diseases bring about destruction of corals to a greater extent than other factors. Investigations on diseases that help identify the factors which cause and manifest the disease will help in the management of diseases¹. We earlier reported partial mortality in the massive coral Porites lutea affected by pink-line syndrome (PLS)²,³. Fungi and a cyanobacterium Phormidium valderianum were isolated from the PLS-affected specimens. The cyanobacterium was identified as the etiological agent in the PLS through Koch’s postulate experiments³,⁴. This communication reports on the cellular changes associated with the PLS-affected corals and the interaction between the host tissue and associated organism.

Coral specimens of P. lutea affected by PLS and healthy ones were collected from a lagoon in Kavarattir island and were transported in sea water to the field laboratory. These specimens were fixed and preserved following the standardized method⁵. Healthy and PLS-affected specimens preserved in 70% ethanol were cut into small pieces of

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