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Pollination biology of *Aristolochia tagala*, a rare species of medicinal importance

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Floral phenology, pollination biology and breeding system were studied in *Aristolochia tagala* Cham. (Aristolochiaceae) grown under *ex situ* conditions. The flower exhibits structural features typical of fly-trap mechanism described for other *Aristolochia* species. Flowers show pronounced protogyny. Stigmas are receptive at anthesis and remain so for 24 h. Anthers dehisce 45–48 h after anthesis by which time stigma receptivity is lost. Chironomid fly (Diptera) is the pollinator. Attracted by the odour and colour of the flower, the flies enter it and are detained in the chamber of the perianth tube (where the anthers and stigma are located) for nearly 50 h. Their escape is prevented by the presence of dense downward-pointing hairs in the perianth tube. The nectaries provide food to the insects. Following anther dehiscence, the thorax of the flies becomes loaded with sticky pollen grains. Hairs on the inner wall of the perianth tube wither and facilitate the exit of the flies. When a fly carrying the pollen load enters a fresh flower, it brings about pollination. Manual pollinations showed that the species permits geitonogamous pollination. The percentage of fruit set in manually pollinated flowers is higher than that resulting from open pollination, confirming that pollination is a limitation for fruit set in the *ex situ*-grown population. Nevertheless, fruit and seed set is sufficiently high for *ex situ* conservation purposes.

Keywords: *Aristolochia* sp., Chironomid fly, geitonogamy, pollination biology.

ARISTOLOCHIA L. is a large genus of the Aristolochiaceae with about 120 species, distributed throughout the tropical and subtropical countries. *Aristolochia tagala*, a climbing shrub is distributed in India, Sri Lanka, China, Malaysia, Burma, Java and Australia, and is a rare medicinal plant. The roots are strongly aromatic and are used to treat snake bites, bone fracture, malaria, indigestion, rheumatism, toothache and various dermatological conditions by Kani tribe of Thiruvananthapuram and Tirunelveli hills[1]. Roots are also used for medicated steam bath 'sudorification'. Leaves are used to treat colic fits and bowel complaints. Due to indiscriminate harvesting of roots for local medicine and trade, the species has become rare in its natural habitat[1]. Saplings collected from natural habitats have been introduced at the Conservatory of the Central Institute of Medicinal and Aromatic Plants (CIMAP) Resource Centre at Bangalore (lat. 13°05 N, long. 77°35 E; altitude 930 m asl). They have established well and are flowering regularly. Each plant produces a large number of flowers (>500).

Adequate knowledge on reproductive biology is essential for conservation, management and recovery of rare and endangered species. To our knowledge, there are no studies on the reproductive biology of *A. tagala*. This communication reports the results of our studies on floral phenology, pollination biology and breeding system of *A. tagala* grown under *ex situ* condition in Bangalore.

Flowers are distinctly stalked, bisexual, zygomorphic with inferior ovary and are produced in axillary cymes (Figure 1 *a*). The perianth consists of three united, tubular, 7–8 cm long, purplish-brown lobes. The perianth tube is 2 mm wide and the inner surface is lined with strigose downward-pointed hairs, which facilitate the entry of flies into the chamber of the flower, but restrict their exit. The perianth tube is swollen into a globose chamber (utricle) in the basal part. The inner surface of the chamber is purplish and bears six dark brown, thick secretory nectaries. The perianth tube terminates into an expanded limb

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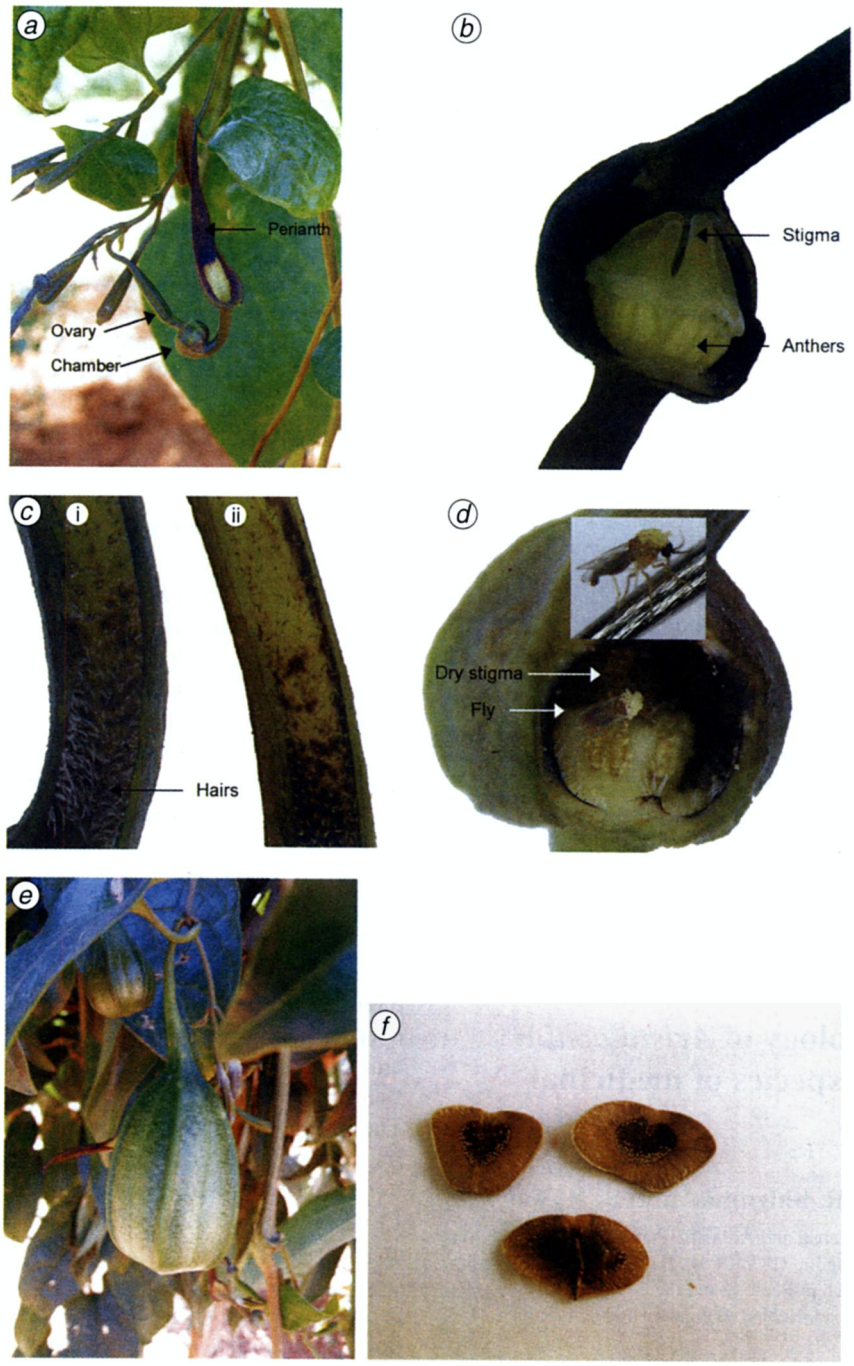

Figure 1. ***a***, Flowering twig of *Aristolochia tagala*, X 0.5. ***b***, Inside view of gynostemium showing arrangement of stigmas and anthers, X 8. ***c***, Inner surface of perianth tube before anther dehiscence (i) and after anther dehiscence (ii), X 5. Note profuse downward-projecting hairs in (i). They have shriveled up in (ii) to allow the fly to escape. ***d***, Perianth chamber opened through a window and photographed to show dehisced anthers and a Chironomid fly with pollen load on its thorax, X 8. (Inset) Fly in greater detail, X 20. ***e***. Mature fruit, X 0.66. ***f***, Winged seeds, X 1.2.

which is deep purplish on the outside and clothed with hairs. The mouth of the perianth tube is creamy-white, 1 cm wide, with few hairs. Stamens, style and stigmas form a united structure in the perianth chamber and are collectively called gynostemium (Figure 1 *b*). The gynostemium bears six stamens on the outer surface and six stigmas on the top. The wall of the chamber has six creamy-white, translucent 'light windows' below the gynostemium.

Table 1. Occurrence of Chironomid flies in perianth chamber during female and male phases of flowers

Hours after anthesis	No. flowers observed	No. flowers with flies (% flowers)	Total no. of flies	Average no. flies per flower
0–24 h (female phase)	44	27 (61.36)	60	2.22
35–50 h (male phase)	35	24 (68.57)	70	2.90

Table 2. Fruit set following manual geitonogamous and xenogamous pollinations

	Geitonogamous pollinations		Xenogamous pollinations	
Hours after anthesis	No. flowers pollinated	Per cent fruit set	No. flowers pollinated	Per cent fruit set
0	15	86.3	19	78.9
01	15	93.3	16	93.7
02	15	86.3	15	93.3
03	15	80.0	16	81.25
04	15	66.6	15	73.3
05	15	73.3	16	62.5
15	15	53.3	15	46.6
20	15	53.3	15	33.3
25	15	33.3	15	33.3
30	15	0	15	0
45	15	0	15	0

Anthesis starts in the afternoon (13.00–18.30 h) with the peak between 14.00 and 15.00 h ($N = 100$). Receptivity of the stigmas was tested through analyses of fruit set following manual pollinations from 0 to 45 h (hourly intervals for the first 5 h and 5 h intervals from 15 to 45 h, $N = 15$ at each interval) after anthesis. Flowers are protogynous and stigmas are receptive at anthesis. The receptive stigmas are yellow, slimy and sticky. Receptivity is maintained for 24 h and then the stigmas start drying. Anthers dehisce extrorsely 45–48 h after anthesis by which time the stigmas are completely dry. The perianth abscises 4–5 days after anther dehiscence. Flowers which do not develop into fruits show drying of the ovary in 2–3 weeks time. Fruit development and maturation are prolonged and take 4–5 months.

A Dipterean Chironomid fly is the pollinator of *A. tagala*. The flies are presumably attracted by the colour and smell of the open flower. They enter the perianth tube and are trapped in the chamber for more than 48 h as the downward-pointed hairs on the perianth tube (Figure 1 *c*(i)) prevent their exit. The flies keep moving about inside the chamber, being attracted by the 'light windows' located below the gynostemium for possible escape. During the first 40 h after anthesis, the perianth hairs present below the gynostemium platform cover the undehisced anthers. Just before anther dehiscence, the size of the chamber enlarges slightly and the hairs surrounding the anthers senesce. The anthers dehisce extrorsely between 45 and 48 h after anthesis, exposing the sticky and yellow pollen clumps. Pollen grains are loaded onto the dorsal surface of thorax of the flies (Figure 1 *d*) during their frequent movement in the chamber in response to the light coming from the windows. By this time, the hairs on the inner surface of the corolla tube undergo senescence and shrivel up enabling the insects to escape (Figure 1 *c*(ii)). Flies carrying pollen on the dorsal surface of their thorax move out between 50 and 53 h after anthesis. When a fly with its pollen load enters another flower, it transfers the pollen nototribically on the gynostemium and brings about pollination. We examined the perianth chambers of flowers during the female ($N = 44$) and male ($N = 35$) phases and recorded the number of flies (Table 1). Over 60% of the flowers in both the phases showed the presence of flies. None of the flowers contained any eggs or larvae.

Breeding system was established by carrying out manual pollinations and recording fruit set. The flowers were bagged before anthesis. Pollinations of bagged flowers were carried out 0–45 h after anthesis by cutting a 0.5 sq cm window in the chamber of the perianth tube and spreading the pollen taken on the tip of a needle onto the stigma. Pollinated flowers were rebagged. Manual autogamic self-pollinations could not be carried out as the stigmas had dried up and lost their receptivity by the time the anthers of the same flower dehisced. Geitonogamous pollination (pollen from another flower of the same plant) and xenogamous pollination (pollen from another plant) were carried out (Table 2). Thirty flowers were bagged and left without manual pollination as additional test for autogamy. One hundred opened flowers were tagged to assess fruit set under open pollination. The duration of fruit maturity was also monitored until seed dispersal.

Bagged flowers without manual pollination did not set fruits, confirming the absence of autogamy in the species. Out of the 100 flowers exposed to open pollination, only 17 set fruits (Figure 1 *e*). However, both geitonogamous and xenogamous pollinations resulted in a significantly

higher fruit set (Table 2). In both types of pollination, the percentage fruit set was high when the flowers were pollinated one and two hours after anthesis. The fruit set gradually decreased until 25 h, after which no fruit set was observed. This was in conformity with the data on stigma receptivity. The fruit size resulting from geitonogamous pollination (4.7 ± 0.09 cm in length and 3.6 ± 0.05 cm in diameter) and xenogamous pollination (4.95 ± 0.06 cm × 3.78 ± 0.07 cm) was uniform. There was not much variation in the size of fruits resulting from open pollination (4.7 ± 0.08 cm × 3.6 ± 0.07 cm). In both types of pollination, each fruit contains over 100 heart-shaped winged seeds (Figure 1*f*).

Most of the species of *Aristolochia* studied so far are reported to be pollinated by saprophagous flies of different families, including Anthomyiidae, Chloropidae, Milichiidae, Phoridae, Sarcophagidae and Syrphidae[2–6]. The nectaries provide nourishment to the flies when they are trapped for over 48 h. Petch[7] and Vogel[8] considered nectar as food for the survival of imprisoned pollinators during captivity rather than a reward. In *A. inflata* and *A. maxima*, the plants provide substrate for larval development and breeding site respectively, to the pollinators[9]. However, in *A. tagala*, no eggs and hatches were observed inside the chamber; hence the flower does not provide a site for breeding and larval development.

Our findings on *A. tagala* indicate that pronounced protogyny precludes autogamy. However, the prevailing breeding system permits geitonogamy. There are a few species of *Aristolochia* such as *A. maxima*, *A. gigantea* and *A. grandiflora*, which are self-incompatible[9,10]. The marked difference in the percentage of fruit set resulting from open pollination and manual pollination in *A. tagala* clearly shows that pollination is a limitation for fruit set under the prevailing conditions in the conservatory. The results are also in agreement with the observations that insects were recorded only in 61.36% of the flowers during the stigma-receptive phase. However, the number of fruits (>100) and seeds (>10,000) produced by *A. tagala* plants even under *ex situ* conditions is quite enormous and offers a distinct advantage for conservational efforts.

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Characterization of a *Citrus exocortis viroid* variant in yellow corky vein disease of citrus in India

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A viroid was isolated and purified from total nucleic acid extract of *Kagzi* lime (*Citrus aurantifolia*) leaves affected by yellow corky vein disease. cDNA of this viroid was cloned in pGEMT-easy vector system and sequenced. *In silico* analysis showed that it consisted of 370 nucleotides. In BLAST analysis the sequence aligned with a different *Citrus exocortis viroid* (CEVd) and thus was tentatively named as yellow corky vein variant of *Citrus exocortis viroid* (CEVd-ycv). This constitutes a report of molecular evidence for occurrence of a *Citrus exocortis viroid* variant from citrus in India. CEVd-ycv showed close phylogenetic relationship with CEVd *Gynura* variants reported from Australia, but was found to be distantly related to *Citrus exocortis viroid* tomato variant (CEVd-t) previously reported from India. The technology for quick and reliable detection of CEVd infecting citrus has been standardized. The tools developed will help identify viroid-free rootstock for use in the budwod certification programme.

Keywords: *Citrus exocortis viroid*, *Kagzi* lime, yellow corky vein disease.

VIROIDS are low molecular weight, infectious, non-encapsidated, self-replicating, circular, single-stranded RNA

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