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Pollination biology of *Curcuma aeruginosa* (Zingiberaceae): An important medicinal plant

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ABSTRACT

The present study reports the details of phenology, floral biology, pollination biology and breeding system of *C. aeruginosa* (Zingiberaceae), in Kerala, India. *C. aeruginosa*is strictly seasonal and bears approximately 7–9 flowers/inflorescence during the peak time of flowering. The nectar is rich in sucrose (77%). The most frequent and efficient pollinator is *Amegilla* sp. (Apidae). Higher percentage of pollen sterility is the cause of poor fruit-set in *C. aeruginosa.*

Keywords : *Amegilla* sp., *Curcuma aeruginosa*, fruitlessness, sterility.

Curcuma aeruginosa Roxb. commonly known as "*Neelakua*" is a natural herbal medicine used for the treatment of diseases of worms, cleaning the blood in women after childbirth, appetite stimulant and can be as foreign drugs for skin diseases (Yuliawati and Hestianah 2010). In Kerala, it is used as a healthy drink for infants, stomach problems, dysentery etc. and also it is an ingredient of various Ayurvedic preparations. The rhizome of *C. aeruginosa* is used medicinally to treat asthma, cough, scurvy, mental derangements, and dysentery. Due to the presence of bioactive components like curcuminoids which are responsible for anti-inflammatory properties, it is also used in wound healing, hypoglycemia, anticoagulant and antimicrobial activities (George *et al*. 2014). For conservation of this species of high medicinal value, studies on pollination biology and breeding system are essential (Moza and Bhatnagar 2007). In the present paper, an attempt has been made in this direction.

MATERIAL AND METHODS

Curcuma aeruginosa has a wide distribution range in Kerala. It is very common throughout the coastal areas and riverine alluvial soil extending up to midlands. During the monsoon season, it is common greenery in coconut and areca nut groves. The study was conducted from 2013 - 2015, at Kunnamangalam, Kozhikode (11.30° N & 75.87° E); Vythiri, Wayanad (11.61° N, 76.21° E), and the Calicut University Campus, Malappuram (11°25´N & 75°50´E), Kerala, India.

Flowering phenology and floral morphology was observed in the field and also in the laboratory with the help of a stereomicroscope (Leica M80). The amount of nectar was determined using micro pipette (10µl) and the concentration of nectar was measured using calibrated hand-held refractometer (WZ 103 BRIX 0-32, China). The components of nectar sugars was analyzed by using HPLC (Shimadzu LC-2010 CHT, Japan).The number of pollen grains/flower, their fertility, viability and pollen germination on the stigmatic surface was studied by various methods described by Shivanna and Rangaswamy (1992). Pollen-ovule ratio was calculated as per the method suggested by Cruden (1977). To check the receptivity of the stigma, cytochemical localization of non-specific esterases was conducted by hydrolysis of the substrate α-napthyl acetate as per Mattson *et al*. (1974). The possibility of wind pollination was studied by hanging vaseline coated slides at various heights on plant. The number of floral visitors, visiting time, foraging nature, foraging hour, time spent in each flower was recorded by using stop watch. The interaction between stigma and insect visitors/pollinators as well as frequency of insect visits were recorded. The insect visitors/pollinators were authentically identified by the kind courtesy of the staff of the Trust for Animal Taxonomy, Zoological Survey of India, Kozhikode.The breeding system determination methods were adapted from Wong and Sun (1999).

RESULTS AND DISCUSSION

Curcuma aeruginosa an important medicinal plant of the family Zingiberaceae is a perennial herb.

Floral phenology—Inflorescence flush occurs prior to pre-monsoon showers and the flowering commence by first week of April. The peak flowering occurs between last week of April and first week of May, and decline in the last week of May followed by vegetative growth which continues till the end of September. By the second week of October, all the aerial parts of the plants perish and underground rhizome survives.

Inflorescences are produced directly from the rhizome before the appearance of leaves and they are laterally positioned (Fig. 1 A), and it takes about 7±1 days for the appearance of first flower on the inflorescence primordia. During the peak flowering period, 7–9 flowers/inflorescence open each day (n=50). The number of flowers produced per each cincinnus is 5±1 (n=50). and total number of flowers produced/plant is 113±12 (n=50). Inflorescence longevity of *C. aeruginosa* is *c.*30 days. Anthesis (Figs. 1 H-K) occurred

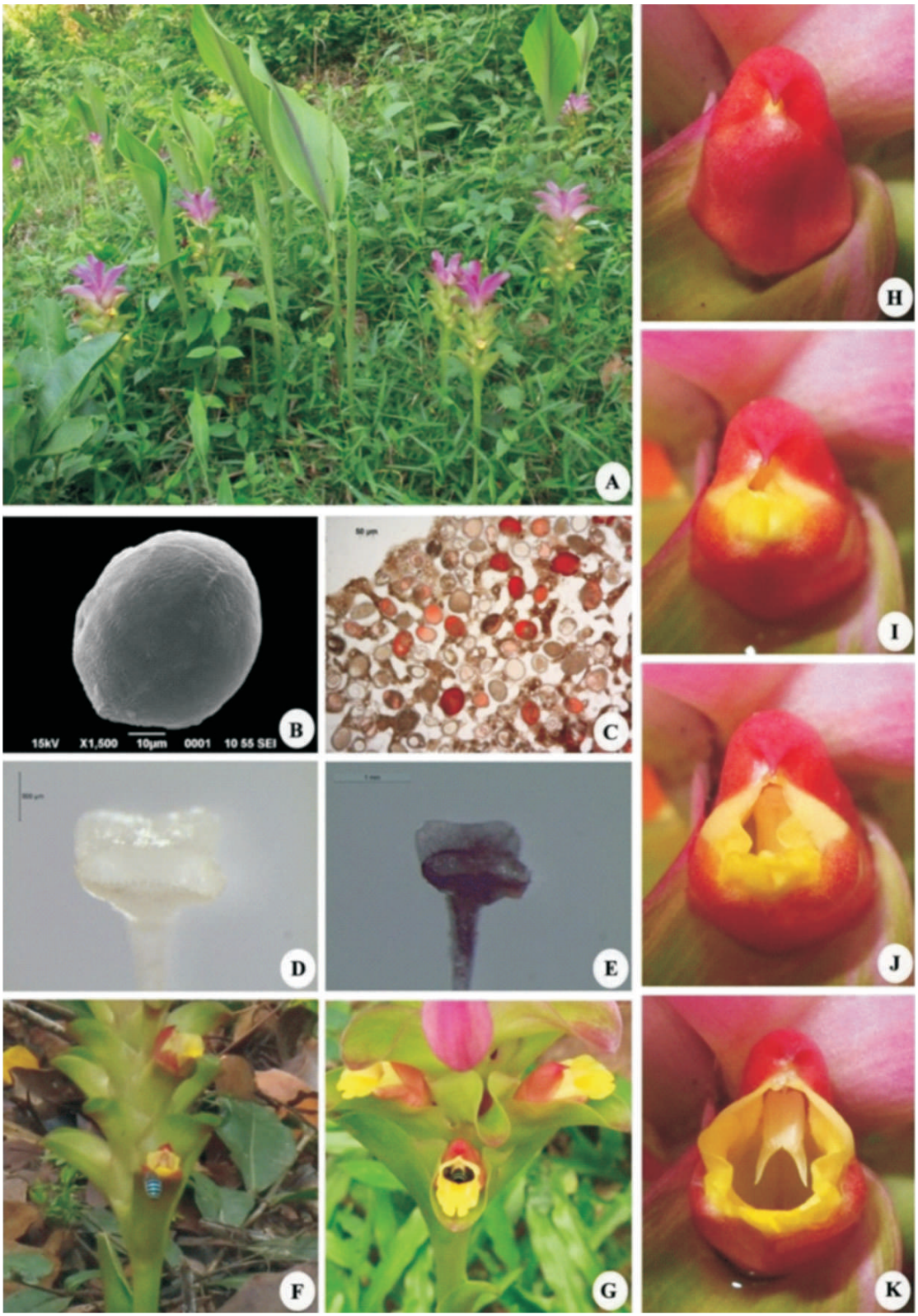

Fig. 1–Some key reproductive features of *Curcuma aeruginosa*. (A) A flowering patch of the plants at the study site (B) A scanning electron micrograph of a pollen grain showing oval to round shape and Psilate exine surface; (C) Pollen viability with TTC test (3 hours after anther dehiscence. Note that only a few pollen grains are viable) (D), Non-receptive Stigma; (E) Receptive Stigma; (F & G) Pollinator - *Amegilla sp.*; (H - K) Different stages of Anthesis (between 06.30 a.m. – 07.30 a.m.) .

between 6.30 a.m. – 7.30 a.m. and the lifespan of a single flower is 24 h. Anthers dehisce soon after anthesis (7.30 a.m. – 8.30 p.m.) by longitudinal slit (from base to top). A flower bud takes 8–10 days from its appearance to full bloom. Floral senescence takes place between 9.00 p.m. – 10.00 p.m.

Flower morphology—The flowers are purplish yellow, equal to or slightly shorter than the bracts, zygomorphic and hermaphrodite. The number of bracts produced per plant is 25±4 (n=50). The corolla tube is *c*. 3–3.3 cm long and purple in colour and lobes are unequal. The labellum is yellow with a deep yellow median band, *c*. 1.5–1.7 *X* 1.8 cm, tip emarginated and it acts as a landing platform for the floral visitors. The anther is *c.* 7 mm long, thecae parallel, without crest, spurred at base, spurs 3 mm long, divergent. Epigynous glands are two in number, 5 mm long, linear, yellowish green. The lateral staminodes are *c*. 1.5 *X* 1 cm, yellow in colour. Ovary is 5 mm in diameter, trilocular, with many ovules with axile placentation. Style is long and filiform. Stigma is bilipped and slightly exserted above the anther lobes (Table 2).

Nectar—Nectar is secreted from the base of the ovary after anthesis and is 30.20±2.94 µl/flower (n=50) by 10.00 and accumulates at the base of corolla tube, thereafter, it decreases gradually. The average sugar concentration in the nectar was 68.10±1.05% (n = 50) at 6.00 p.m. and it is sucrose dominant with 77% sucrose, 16% glucose and 7% fructose.

Pollen grains—The pollen grains measure 68.16 ± 1.42 µm (n = 100) across, oval to round in shape and psilate or unsculptured type (Fig. 1 B). Testing pollen grains with Sudan black B and I_2KI solution indicated the presence of lipids and starch. There were 8949±162 pollen and 60±1 ovules/flower (n=50). Hence, the pollen ovule ratio was 149 indicating allogamous nature of flowers (Cruden 1977). The maximum pollen viability under natural condition at 10.00 a.m. was 17.40 ± 0.42% nearly 3 hour after anthesis (Fig. 1 C). Pollen viability in flowers kept in petri dishes lined with wet filter paper, at room temperature, -20°C, 0°C and 4°C and after 4 hours quickly declined and viability is completely lost within 4 hours. Maximum viability (3.9±0.73%) in flowers stored at -20°C indicated that pollen viability fail to improve by storing them under low temperature.

Table 1-Pollination treatments used to determine breeding system of *C. aeruginosa*

Sl. No.	Breeding system test	Number of flowers observed	Flowers bagged	Treatment	Pollen source	Fruit Set (%)
1	Apomixis	50	Yes	Emasculate	No pollination	0
2	Spontaneous autogamy	50	Yes	None	Same flower	0
3	Induced autogamy	50	Yes	Emasculate	Same flower	0
4	Artificial geitonogamy	50	Yes	Emasculate	Different flower, same plant	0
5	Artificial xenogamy	50	Yes	Emasculate	Different population	0
6	Control	50	No	None	Open pollination	0

Table 2- Floral characters of *C. aeruginosa*

Sl. No.	Floral characters	Observations
1.	Flowering period	April & May
2.	Flower type	Zygomorphic, Hermaphrodite
3.	Flower colour	Purplish Yellow
4.	Odour	Present
5.	Nectar amount (max.)	30.20 ± 2.94
6.	Nectar concentration (max.)	68.10 ± 1.05
7.	Anthesis time	06.30 a.m. – 07.30 a.m.
8.	Anther dehiscence time	07.30 a.m. – 08.30 a.m.
9.	Anther dehiscence mode	Through Longitudinal slit
10.	Number of anthers / flower	1
11.	Mean number of pollen grains / flower	8949± 162
12.	Mean number of ovules / flower	60± 1
13.	Pollen - ovule ratio	149: 1
14.	Pollen type	Psilate
15.	Pollen size	68.16 ± 1.42
16.	Pollen shape	Oval to round
17.	Stigma type	Wet type
18.	Stigma shape	Cup shape
19.	Pollen viability [%] (max.)	17.40 ± 0.42
20.	Flower closing time	9.00 p.m. – 10.00 p.m.

Table 3–Flower visitors and Pollinator of C. aeruginosa

Sl. No.	Name of taxa with family	Visiting time	Foraging nature	Foraging hours	Time spent in each flower	Stigma touch	Frequency of visit
1.	*Amegilla sp.*	Day	Nectar + Pollen	8.00 a.m.-5.00 p.m.	1-2 seconds	+++	High
2.	*Apis dorsata Apidae*	Day	Nectar + Pollen	3.45 p.m.-5.15 p.m.	2-3 seconds	+++	Intermediate
3.	*Apis sp. Apidae*	Day	Nectar + Pollen	5.10 p.m.-5.20 p.m.	2-4 seconds	++	Intermediate
4.	*Halictus sp.*	Day	Nectar + Pollen	12.45 p.m.-5.15 p.m.	3-4 seconds	++	Intermediate
5.	*Ceratina sp.*	Day	Nectar + Pollen	10.15 a.m.-3.30 p.m.	3-4 seconds	++	Intermediate
6.	Beetle Nitidulidae	Day	Anther	9.30 a.m.-10.00 a.m.	1-2 minute	-	Intermediate
7.	Beetle 2	Day	Nectar + anther lobes	8.20 a.m.-8.30 a.m.	4-5 seconds	-	Intermediate
8.	Beetle 3	Day	Nectar	3.15 p.m.-3.20 p.m.	3-5 seconds	-	Intermediate
9.	*Thomisus projectus* Thomisidae	Day	Insects	12.55 p.m.-1.00 p.m.	2-5 seconds	-	Intermediate
10.	Wasp 1	Day	Nectar	4.30 p.m. -4.45 p.m.	2-4 seconds	-	Low
11.	*Thomisus lobosus* Thomisidae	Day	Insects	4.45 p.m.-5.15 p.m.	3-5 minute	-	Intermediate
12.	*Crematogaster sp* Formicidae	Day	Pollen	9.00 a.m.-9.40 a.m.	3-5 minute	-	Intermediate
13.	*Camponotus parius* Formicidae	Day	Pollen	12.50 p.m.-1.00 p.m.	3-4 seconds	-	Intermediate
14.	*Crematogaster sp* Formicidae	Day	Pollen	10.00 a.m.-10.20 p.m.	3-4 seconds	-	Low
15.	*Camponotus sp.* Formicidae	Day	Nectar	4.30 p.m.-4.45 p.m.	3-4 minute	-	Low
16.	*Drosophila melanogaster* Drosophilidae	Day	Pollen	8.30 a.m.-5.30 p.m.	1-2 minute	-	High
17.	*Udas pesfolus* Hesperidae	Day	Nectar	9.15 a.m.-10.30 a.m.	1-2 minute	-	High
18.	*Pelopidas mathias* Hesperidae	Day	Nectar	11.20 a.m.-11.30 a.m.	1-2 minute	-	High
19.	*Notocrypta curvifascia* Hesperidae	Day	Nectar	3.00 p.m.-3.15 p.m.	5-7 seconds	-	Intermediate
20.	*Camaricus formosus* Thomisidae	Day	Insects	4.00 p.m. – 4. 45 p.m.	1-3 minutes	-	High
21.	Mosquito	Day	Plant juice	9.00 a.m. – 2. 45 p.m.	1-3 minutes	-	Intermediate
22.	*Iphita limbata* Pyrrhocoridae	Day	Plant juice	1.00 p.m. – 5. 45 p.m.	2-4 minutes	-	Intermediate
23.	*Letana sp.* Tettigonidae	Day	Anther, Flower, leaf	11.00 a.m. – 3. 15 p.m	2-4 minutes	-	Intermediate

Stigma touch - +++: very good, ++: good, +: poor, - : no
Frequency of visit – high (5-30 visits / day), intermediate (1-5 visits/day), low (<1visit /day.

Pistil—During the receptive phase, the stigma produced exudates at the tip and the stigma show maximum receptivity as marked by the presence of enzyme esterases on their surface between 4.00 to 5.00 pm (Fig. 1E) as reported earlier in other members of Zingiberaceae (Aswani *et al*. 2013, Aswani and Sabu 2015a, Aswani and Sabu 2015b).

Pollination biology—Fresh open flowers offer both pollen and nectar to the visitors which start foraging soon after anthesis in the morning, but their visitation rates declined by 15 h. Similar floral visitors were recorded at all the study sites (Vythiri, Wayanad and Kunnamangalam, Kozhikode). Floral visitors were *Amegilla* sp*., Apis dorsata, Apis* sp., *Halictus* sp., ants (*Camponotus parius*, *Camponotus* sp.and *Crematogaster* sp.), butterflies (*Udaspes folus*, *Pelopidas mathias* and *Notocrypta curvifascia*), *Drosophila melanogaster*, spiders (*Thomisus projectus* and *Camaricus formosus*), *Iphita limbata*, *Letana* sp. and wasp (Table 3). Among these, the effective pollinators were *Amegilla* sp*., Apis* spp., *Ceratina* sp.and *Halictus* sp. And they prefered to visit on sunny days while the visitation rates during the rainy days declines. The most frequent and effective pollinator at all the study sites is *Amegilla* sp. (Figs. 1F,G), and during its single visit it foraged 8–16 flowers. The entry of the insect in the flower, anthers spur effectively supports in pollination. In order to understand the pollination efficiency under natural conditions, stigmas were collected 24 hours after anthesis and observed under a microscope. Out of 100 flowers observed, pollen grains were present on stigmas of only 74% of flowers. On the other hand,

in order to record the pollination efficiency of flower visitors, the flowers were excised soon after their first visit and examined the stigma for pollen deposition under a stereomicroscope. The results of these experiments indicated that *Amegilla* sp. shows maximum pollination efficiency as compared to other visitors.

Breeding system—The flowers of *C. aeruginosa* fail to show fruit-set in the natural population (Table 1). Present observation is confirmed by those of Sastrapradja and Aminah (1970). They reported that, *Curcuma* sp. in spite of prolific flowering in Indonesia fail to produce fruits. Preset hand pollinated experiments with the emasculated and bagged flowers failed to show fruit-set by selfing, geitonogamy and xenogamy. The stigma of *C. aeruginosa*, protruded well above the anther in quest of pollen. Protruding stigma in several male sterile plants have been reported (Chauhan and Singh 2003). Various insect vectors forage the flowers and come in contact with the stigma, but there is no fruit-set. Various techniques used for artificial pollination also failed to produce fruits in *C. aeruginosa.* It is therefore, concluded that significantly poor pollen viability is the cause of fruitlessness in *C. aeruginosa.*

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