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Reproductive biology of dancing girl ginger, *Globba schomburgkii* (Zingiberaceaeae)

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ABSTRACT

Globba schomburgkii (Zingiberaceaeae) is an ornamental ginger with a very conspicuous floral display, but almost no fruit set under field condition. The pollination biology of *G. schomburgkii* was studied to determine the pollination system and the reason for fruitlessness. Studies were conducted over two consecutive years (2016 and 2017) at Calicut University Campus, Kerala, India. Phenological studies indicated that the species shows a regular flowering season. Flowers are zygomorphic and hermaphrodite. The flowers are mainly visited by *Amegilla zonata*, which is the effective pollinator. The study confirmed that low percentage of pollen viability is responsible for the fruitlessness and the plant is vegetatively propagated, bulbils being the main propagules.

Keywords : *Amegilla zonata*, Bulbils, *Globba schomburgkii,* Pollination biology.

Globba (Zingiberaceae–Zingiberales) consists of *ca.* 100 species of small perennial herbs from East Asia and Malaysia (Box and Ruddell 2005). Flowers of *Globba* are striking orange, yellow, purple or white, contrasting with the often green inflorescence bracts (Endress 1994, Takano and Okada 2003). They possess extraordinarily specialized morphology, even within the context of Zingiberaceae, in which there is invariably a single fertile stamen (the adaxial stamen) and two sterile inner androecial members are fused and petaloid, forming an abaxial labellum (Kirchoff 1997, 1988a, b, Endress1994, Box and Ruddell 2005).

G. schomburgkii is an ornamental ginger, commonly known as dancing girl ginger. It is distributed mainly in Eastern India, Myanmar, Thailand, Vietnam, and South China. Each stem produces a terminal inflorescence. Beautiful golden yellow flowers are borne in a pendulous raceme or thyrse (Sabu 2006).

Pollination biology is poorly known in *Globba* and other Zingiberaceae (Endress 1994, Ippolito and Armstrong 1993). *G. schomburgkii* produces mass flowers and pollination take place, but there is no fruit set. Pollination can be the first factor limiting fruit production (Schemske 1980, Howell and Roth 1981, Arista *et al.* 1999), and the study of the reproductive ecology of flowering plants is important for determining barriers to seed and fruit set (Gao *et al.* 2006). So we decided to study the reproductive biology of *G. schomburgkii*. Here we present the results of our investigations, which addressed the following questions concerning the reproductive biology of *G. schomburgkii*: (i) what are the flower morphology, floral phenology, pollen biology of *G. schomburgkii*? (ii) What are the barriers to fruit set? (iii) What are the main visitors to *G. schomburgkii*, and are they effective pollinators?

MATERIALS AND METHODS

Study plant—The study was conducted on different populations of *G. schomburgkii* (Fig.1A) at Calicut University Botanical Garden (CUBG) in Kerala, India (11°25´N & 75°50´E) during 2016 and 2017.

Flower morphology—Floral morphology was studied in the field and also in the laboratory with the help of a stereomicroscope (Leica M80). The measurements of the floral parts were taken with the help of scale. By direct visual observation, the colour of the flower was determined. The presence of smell of these flowers was detected by keeping some flowers in bottles for two hours.

Flowering phenology—Flowering phenology was observed in the field in two flowering seasons in 2016 and 2017. Frequent visits were made to the study sites to observe the flowering season. Regular observations were carried out from the period of inflorescence initiation to the period of fruit maturation. Anthesis and anther dehiscence was observed in the field using hand lens, following the method of Reddi and Janaki Bai (1981), Mathur and Mohan Ram (1986), Ramasubbu *et al*. (2009).

Nectar volume and sugar concentration—To estimate the volume of nectar and sugar concentration flowers were randomly selected from different plants and bagged just before opening to prevent floral visits. They were excised at hourly intervals (N=50) and the amount of nectar was determined using micro pipette (10 µl) and the concentration of nectar by using calibrated hand refractometer (WZ 103 BRIX 0 – 32, China).

Pollen biology—In Zingiberaceae pollen morphology is determined by mounting pollen grains directly in glycerine jelly after killing and fixing fresh pollen grains in 70% alcohol and washing it in distilled water (Mangaly and Nayar 1990), because acetolysis dissolved the pollen completely in majority of Zingiberaceous taxa. Photomicrographs of the sample were taken at appropriate magnification using Zeiss Axiolab A, stereomicroscope. The average size of pollen grains was measured from a random sample of 100 pollen grains in each species by using Axiovision 4.8 software. The biochemical

Fig.1-*Globba schomburgkii.* A. Habit. B. Inflorescence. C. Single flower. D. Floral development. E. Winged anther. F. Stigma. G. Pollen viability. H. Pollen germination. I. *Eurema hecabe.* J - L. Pollinator – *Amegilla zonata.* M. *Udaspus folus.* N. Bulbils. O. Seedlings.

analysis of pollen grains was done using IKI, sudan black B and Coomassie brilliant blue R for the detection of starch, lipid, and protein, respectively. The viability of pollen grains was assessed by Triphenyl tetrazolium chloride test (Shivanna and Rangaswamy 1992). Pollen-ovule ratio was calculated as per the method suggested by Cruden (1977). The effect of organic and inorganic nutrients on *in vitro* pollen germination and pollen tube elongation were studied using Brewbaker and Kwack's medium, solutions of boric acid (100 - 500 µg/l), calcium nitrate (25 - 500 µg/l), magnesium sulphate (25 - 500 µg/l), potassium nitrate (25 - 500 µg/l) and glucose solution (1 - 40 µg/l). The percentage of pollen germination and pollen tube elongation were observed under Lab. A1 ZEISS Axiolab compound microscope (Zeiss, Germany).

Stigma receptivity—To check the receptivity of the stigma, cytochemical localization of non-specific esterases was conducted by hydrolysis of the substrate α-napthyl acetate (Mattson *et al*. 1974) and also using hydrogen peroxide (H_2O_2) reaction after Scribailo and Usher (1984).

Pollination biology—The number of floral visitors, visiting time, foraging nature, foraging hour, time spent in each flower was recorded by using stop watch, stigma touch by insects and frequency of visit were recorded during 30 days per each flowering period. The visitors were trapped by a net and transferred them into a bottle containing a piece of filter paper dipped in ethyl acetate. Collected visitors were transferred to a glass slide and observed under a microscope. Pollination efficiency of different pollinators was studied by observing the pollen loads on different body parts according to the procedure given by Kearns and Inouye (1993). To check pollen load on stigmatic surface, stigmas (n = 50) were collected after each visit of insects and observed under a microscope.The insects were identified by an entomologist, at the Trust for Animal Taxonomy, Zoological Survey of India, Kozhikode.

Breeding system—The breeding system determination methods were adapted from Wong and Sun (1999). Breeding behaviors (autogamy, geitonogamy and xenogamy) was tested using controlled pollination studies in emasculated and bagged flowers. Self- and cross-pollination experiments were performed by dusting pollen obtained from freshly dehisced anthers on the receptive stigma. The pollinated flowers were re-bagged and observed periodically for fruit formation.

Statistical analysis—Data from each period of observation were analysed using IBM SPSS Statistics 20 and results were presented as mean ± standard error (SE).

RESULTS AND DISCUSSION

Flower morphology—The inflorescence is terminal, decurved, bearing many slender branches, bearing 4-5 flowers in a cincinnus. The inflorescence is a thyrse, the upper part bears flowers and the lower part bears vegetative propagules (bulbils). In the upper (fertile) region, each bract subtends a short inflorescence (cincinnus) in a zig-zag arrangement. This is similar in other *Globba* spp. reported earlier (Box and Rudall 2006). The flowers are golden yellow in colour (Fig.1C).The floral tube is long. The labellum is triangular with divergent lobes, golden yellow in colour with orange patch at the centre. Flowers produce a single fertile stamen (Fig.1E); labellum is the prominent landing platform for small insects. Anther is small with two spreading, narrowly triangular appendages on each side on a long arching filament, like a bow. The characteristic anther wings are believed to function as levers, which allow the anther to be oriented into a favourable position for the transfer of pollen; their lateral position may orient the anther correctly if the flower is approached laterally by the pollinator (Endress 1994). The pistil was well demarcated into ovary, style and stigma. Ovary was inferior and unilocular with many ovules borne on parietal placenta. Style was slender and long. The stigma (Fig.1F) was colourless, funnel-shaped with ciliate margin composed of stiff bristles and clasped between the enlarged thecae of stamen. Floral characters are incorporated in Table 1.

Table 1– Floral characters of G. schomburgkii

S. No.	Floral characters	Observations
1.	Flowering period	June to August
2.	Flower type	Zygomorphic, Hermaphrodite
3.	Flower colour	Golden yellow with orange patch at the center
4.	Odour	Present
5.	Nectar amount	67.6±2.5µl
6.	Nectar concentration	5.30 – 6.30 a.m.
7.	Anthesis time	10.30 – 11.00 a.m.
8.	Anther dehiscence time	Longitudinal slit
9.	Anther dehiscence mode	1
10.	Number of anthers / flower	2647±112
11.	Mean number of pollen grains / flower	25±3
12.	Mean number of ovules / flower	106.1
13.	Pollen - ovule ratio	Granulate
14.	Pollen type	54.82±3.43µm
15.	Pollen size	Round or spherical
16.	Pollen shape	Wet type
17.	Stigma type	13.27±2.35
18.	Pollen viability (%)	28±4
19.	Number of bulbils	6.00 – 7.00 p.m.
20.	Flower closing time	

Flowering phenology—Under favourable climatic conditions of Calicut University Botanic Garden, *G. schomburgkii* flowers June to August. It took 17 - 20 days to develop young primordia of the inflorescence into a complete inflorescence (Fig.1B). The peak flowering period was July. During the peak flowering period 4 – 6 flowers bloom on each inflorescence. One inflorescence of *G. schomburgkii* lasts about 1 – 2 months. Anthesis occurs between 5.30 - 6.30 a.m. and anther dehiscence was observed between 10.30 a.m. –

11.00 a.m. The lifespan of a single flower is 1 day. Similar observations were found in other genera of Zingiberaceae, previously reported by various authors (Cui *et al.* 1995, 1996, Li *et al.* 2001a, b, 2002, Zhang *et al.* 2003, Wang *et al.* 2005a, b, Aswani *et al.* 2013, Aswani and Sabu 2015).

Nectar volume and concentration—Floral nectaries were epigynous. Nectar secretion started soon after anthesis and accumulated gradually and reached about 67.6±2.50 µl at 9.00 a.m. (highest volume of nectar). Then the volume gradually declined to 23.1±1.65 µl at 6.00 p.m. The highest sugar concentration (31.07±1.24 %) was observed at 6.00 p.m. The results are incorporated in the Table 2.

Table 2– *G. schomburgkii :* Nectar volume and sugar concentration

S. No.	Time of observation	Average volume of nectar (µl)/ flower ± S.E.	Average sugar concentration of nectar (%)/ flower ± S.E.
1	7 a.m.	48.4±0.51	8.9±1.11
2	8 a.m.	60.9±0.93	6.4±1.24
3	9 a.m.	67.6±2.50	4.2±1.9
4	10 a.m.	62.2±0.94	6.1±1.6
5	11 a.m.	56.1±0.62	7.4±1.4
6	12 p.m.	44.1±0.14	10.5±1.3
7	1 p.m.	37.4±0.71	16.4±0.9
8	2 p.m.	23.9±0.94	22.1±1.24
9	3 p.m.	19.5±0.41	26.5±1.67
10	4 p.m.	11.3±0.55	27.9±1.15
11	5 p.m.	7.3±0.86	29.4±1.44
12	6 p.m.	3.5±0.46	31.07±1.24

Pollen biology—Pollen grains were spherical with a diameter of 54.82±3.43 µm and granulate type. A flower produced an average of 2647±112 pollen grains and 25±3 ovules (n= 50). Hence, the pollen-ovule ratio was calculated as 106: 1. Pollen grains stained with I.KI solution, Sudan Black and Coomassie Brilliant Blue, indicated the presence of starch and lipid, but protein was absent. Fresh pollen grains showed maximum viability (13.27±2.35%) at the time of anthesis (Fig. 1G), thereafter the viability decreased gradually. Germination potential was very poor in *G. schomburgkii*. Brewbaker and Kwack's medium had more effect on pollen germination than sucrose solution. It has been observed that 47.16±1.3% of the pollen grains germinated with a mean of 492.43±3.6 µm long pollen tube (Fig.1H) observed after six hour incubation in Brewbacker and Kwack's medium. But in Sucrose solution only 22.91±0.8% of pollen grains germinated with a mean of 112.47±2.6 µm long pollen tube.

Stigma receptivity—Receptivity of stigma is the critical factor for successful completion of the post-pollination events. The enzyme peroxidases were present in receptive stigmatic surface. When we treated with hydrogen peroxide oxygen bubbles were evolved from the surface. The stigma showed maximum bubble activity (48±4 bubbles/ minute) during 09.00 a.m.to 11.00 a.m. The enzymes esterases were also present in receptive stigma surface, confirmed by cytochemical localization of stigmatic esterases using α-naphthyl acetate. This result was also support the hydrogen peroxide test.

Pollination biology—The flowers of *G. schomburgkii* provided both nectar and pollen as rewards to the visitors. The insects visiting the flowers were *Amegilla zonata* (Figs.1 J, K & L) a blue-banded bee species (Apidae) and butterflies such as *Eurema hecabe* (Pieridae) (Fig.1I) & *Udaspus folus* (Hesperidae) (Fig.1M). Floral visits started with the opening of flowers and anther dehiscence. Visits to the flowers of *G. Schomburgkii* were dominated by *A. zonata* during morning, noon and evening times of the day, so this species can be considered as the most frequent and effective pollinator. When these bees entered the flower, the pollen grains were dusted on their dorsal surface. After nectar collection these bees returned and deposited pollen grains on the receptive stigmatic surface of a different flower, which facilitates cross pollination. Winged anther lobes effectively help bees for pollination. A single bee generally visited three to five flowers/inflorescence per single visit to the plant and, although the time interval between visits varied, each bee spent 2-4 seconds on a single flower. The same butterflies made 3-4 visits/ flower with each visit of 15-20 seconds. They landed on the labellum and inserted their proboscis into the corolla tube and collected the nectar. These were merely nectar robbers. Since no pollen was deposited on any of the Vaseline coated slides hung near the population, it was concluded that wind has no role in the pollination of this species. In accordance, no reports have been published on wind pollination in Zingiberaceae (Aswani *et al.* 2013).

Breeding system—There was no apomixis, as none of the emasculated and bagged flowers set fruit. To determine if the species is self-incompatible both self and cross pollinations were carried out. Bagged flowers without manual pollination did not set fruits, confirming the absence of autogamy in the species. Bagged flowers were pollinated by pollen grains from another flower of the same plant or with pollen grains from a different plant, but these pollination experiments failed to set fruit either. These results confirmed that this plant produced no fruits and seeds. Low percentage of pollen viability was the main cause of fruitlessness in *G. schomburgkii.*

G. schomburgkii was mostly dependent on bulbils (Fig.1N) for its reproduction. Bulbils are the vegetative propagules of *Globba* located towards the bases of the inflorescences. Each bulbil is pale (almost white), spherical with a pitted surface, and occurs in the axil of a bract. An average of 28±4 bulbils was present. These bulbils germinated in next season. Bulbil production allows herbaceous plants to grow vigorously and establish rapidly, and may alleviate competition for pollinators. Bulbils are rare in tropical and subtropical plants (Wang and

Cronk 2003), but *Globba* is unusual in being a tropical species that invests heavily in vegetative reproduction by means of bulbils (Box and Rudall 2006).

The present work confirmed that *G. schomburgkii* is a vegetatively propagated plant, bulbils being the main propagules. *Amegilla zonata* was the main and most effective pollinator. Low percentage of pollen viability is in the background of fruitlessness. Similar results were obtained in other members of Zingiberaceae (Aswani and Sabu 2017).

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