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Research article

REPRODUCTIVE BIOLOGY OF *ETLINGERA ELATIOR* (JACK.) R. M. SM. ORNAMENTAL TORCH GINGER

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ABSRACT: The reproductive biology of *Etlingera elatior* (Jack.) R. M. Sm. (Zingiberaceae), torch ginger was studied at Calicut University Botanical Garden, Kerala. This study seeks to determine the flowering phenology, pollen viability, stigma receptivity, identify floral visitor, identification of pollinators and evaluate pollination efficiency. *E.elatior* flowers all year round, with a peak flowering January to April. During the peak flowering period 18 – 24 flowers bloom on each inflorescence. One inflorescence of *E. elatior* has a life of 3 – 4 months. Anthesis occurs between 5.30 - 6.30 a.m. and the lifespan of single flower is 1 day. *Nectarinia asiatica*, *Nectarinia zeylonica* (Male & Female) and *Tetragonula irridipennis* are most efficient pollinators. Percentage of fruit set in artificial cross pollination is higher than that resulting from natural pollination, which strongly indicates that some external agents are required for effective pollination. It is the reason for low fruit set in *E. elatior*.
Keywords: *Etlingera elatior*, *Nectarinia zeylonica*, Pollination efficiency, Torch ginger, Zingiberaceae

INTRODUCTION

The family Zingiberaceae are the largest family of the order Zingiberales and one of the ten largest monocotyledonous families in India. It consists of about 53 genera and more than 1200 species. They are distributed mainly in tropics and subtropics with centre of distribution in the Indo-Malayan region, extending through tropical Africa to Central and South America [1]. The word ginger refers to the members of the family Zingiberaceae. Many species are economically important as source of food, spice, medicine and ornamentals. India has rich diversity of Zingiberaceous plants, perhaps 168 of the world taxa occur in India. The over exploitation of rhizomes for medicinal use and food have placed many taxa under severe threat. The Zingiberaceous plants are annual or perennial rhizomatous herbs. Zingiberaceae are characterised by specialized zygomorphic, long-tubed flowers. It is one of the most diversified plant groups in the tropics. Morphological characters of Zingiberaceous flowers are thought to have coevolved along with their pollinators [2]. Reproductive biology of different plants and ask how interactions of certain pollinators with plants and their behaviour on flowers influence the morphology of reproductive organs or the flowering phenology, and how this can influence fruit and seed production in relation to the whole reproductive cycle. *E. elatior* is an important ornamental plant of the family Zingiberaceae. To our knowledge, there are no studies on the reproductive biology of *E. elatior* have been carried out. The present paper reports the results of our work on the phenology, floral biology, pollen biology, pollination mechanism, pollination biology, breeding system and seed germination in *E. elatior*.

MATERIALS AND METHODS

Study site

The study was conducted from November 2011 to October 2012, in the Calicut University Botanical Garden (CUBG) in Kerala, India.

Study Plant

E. elatior is an ornamental ginger of the family Zingiberaceae. It is distributed throughout south eastern Asia, New Guinea, the Bismarck Archipelago, the Philippines, Australia (Queensland), and Polynesia. It is a perennial herb. The large, torch like up to 1 m tall flower stalks emerge from fleshy underground rhizomes. The leaves are green, glabrous, lanceolate in shape and up to 81cm long. The psuedostems (formed by the leaf sheaths) emerge from underground rhizomes and are tall and arching.

The inflorescences have waxy, red to pink edged bracts and are pine cone-shaped with a skirt of larger bracts. The individual flowers emerge from between the colourful bracts and have a dark red labellum (lip petal) with a bright yellow margin. The flowers are followed by green to reddish fruit. Fruit is a capsule with many black seeds. It is widely cultivated in tropical countries and used as spice, food flavouring and also used as cut flowers [3]. Flowering phenology and floral morphology was observed in the field and also in the laboratory with the help of a stereomicroscope (Leica M80). To estimate the amount of nectar, 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 (10µl) and the concentration of nectar was measured using calibrated hand refractometer. The number of pollen grains/flower, their fertility, viability was studied by various methods described by [4]. Pollen-ovule ratio was calculated as per the method suggested by [5]. Stigma receptivity determined by using hydrogen peroxide (H_2O_2) reaction after [6]. 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. The insects were identified by an entomologist, at the Trust for Animal Taxonomy, Zoological Survey of India, Kozhikode. Breeding behaviour (autogamy, geitonogamy & 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 [4].

RESULTS

Flowering phenology

Under favourable climatic conditions of Calicut university Botanic Garden, *E. elatior* flowers throughout the year. The inflorescence was produced directly from the rhizome. It takes 40 - 52 days to develop young primordia of the inflorescence in to a complete inflorescence (Fig. 1a). The height of inflorescence varied according to plants and also with season. The average height was 30.92 ± 2.01cm. The flowers are bright red in colour with yellowish margin and have an elongated corolla tube. Flowers produce a single fertile stamen; labellum is the prominent landing platform for small insects. *E.elatior* flowers all year round, with a peak flowering January to April. During the peak flowering period 18 – 24 flowers bloom on each inflorescence. One inflorescence of *E. elatior* lasts about 3 – 4 months. Anthesis occurs between 5.30 - 6.30 a.m. and the lifespan of single flower was 1 day.

Flower morphology

Inflorescences are densely congested with sterile bracts and fertile bracts. The fertile bracts have a single bracteole and flower, inner ones are narrow and outer ones are broader and becoming transitional with involucral bracts. The flowers are bright red with yellow margin in colour (Fig. 1b). The bracteoles are tubular, two to three dentate and persistent. The floral tube is about as long as or shorter than calyx. The lobes are usually shorter than the tube. The calyx is tubular and often split on one side. The labellum is entire or emarginate, short, or with a conspicuous central lobe and lateral lobes that may overlap the stamen. The stamens (Fig. 1f) are basally adnate to the filament forming a tube above the insertion of the corolla lobes. The lateral staminodes are absent or merely rudimentary. The filament was very short or absent, anther is often emarginated and nearly erect or at a sharp acute angle with respect to the filament.

Nectar volume and concentration

Nectar is a very important floral reward secreted by floral nectaries. The amount of the reward presented to a flower visitor is an important factor which determines the behaviour of the visitor. The amount of reward offered to a flower visitor also depends on the concentration of sugar in the nectar. Nectar secretion starts soon after anthesis and accumulates gradually and reached about 47.6±1.40 µl by 9.00 a.m. (highest volume of nectar). Then the volume gradually declined to 27.1±1.45 µl on 6.00 p.m. The highest sugar concentration (26.02±1.51 %) occurs on 9.00 a.m., and there after the concentration also declined, and reached to 22.05±1.43 % on 6.00 p.m.

Pollen grains

Pollen grains 89.53±4.55 µm in size round or spherical and psilate or unsculptured type. The mean number of pollen grains 6317±32 and pollen-ovule ratio 70: 1. Pollen grains stained intensely with I_2KI solution, Coomassie Brilliant Blue and Sudan Black indicating the presence of starch and protein but lipid is absent. Acetocarmine - Glycerine staining technique indicate that a high percentage of pollen grains (86.6±1.6 %) are fertile in *E. elatior*. Fresh pollen grains at the time of anthesis showed maximum (90.52±2.35 %) viability (Fig. 1h) there after the viability decreased steadily and reached 8.14±1.43 % on 6.00 p.m. Germination potential was very poor in *E. elatior*. Brewbaker and Kwack's medium has more effect on pollen germination than sucrose solution. It has been observed that 33.86±1% germinated pollen with a mean of 512.40±5 µm long pollen tube (Fig. 1i) occurred after six hour incubation in Brewbacker and kwack's medium.

Pistil

The pistil was well demarcated in to ovary, style and stigma. Ovary barrel-shaped, superior and trilocular, each locule bears many ovules borne on an axile placenta. Style filiform and dark purple in colour, stigma dark red and club-shaped (Fig. 1g). Receptivity of stigma is the critical factor for successful completion of the post pollination events. Hydrogen peroxide was applied to determine whether the stigma was receptive when the anthers dehisce and the presence of a bubble of Oxygen is an indicator of stigma receptivity. In *E. elatior*, stigma showed maximum bubble activity 64.61±0.70 bubbles/ 1minute on 6.00 a.m. soon after anthesis. Thereafter the receptivity of the stigma decreased steadily and reached 15.69±2.98 bubbles/ minute on 6.00 p.m. the flower start to senescing.

Pollination biology

The flowers of *E. elatior* provide both nectar and pollen as rewards to the visitors. The insects visiting the flowers are birds like *Nectarinia asiatica*, *N. zeylonica* (Female) (Fig. 1j), *N. zeylonica* (Male) (Fig. 1k) and *Centropus sinensis*, *Tetragonula irridipennis*, *Ceratina* sp., ants like *Paratrichina sp.* and *Oecophylla smaragdina*, *Drosophila melanogaster*, spiders, cockroach and butterflies. These start floral visits with the opening of flowers and anther dehiscence. Visits to the flowers of *E. elatior* were dominated by birds during morning, noon and evening times of the day. The most frequent birds visiting the flowers were two species of *Nectarinia*. The birds land on the inflorescence and push their beak in to the flowers for nectar collection, which helps in cross pollination. A single bird generally visited one to five flowers/inflorescence and, although the time interval between visits varied, each bird spent 2-4 seconds on a single flower. When visiting the flowers, *Tetragonula irridipennis* (Fig. 1c,d) landed on the labellum near the anther and walked shortly into the corolla tube. It forages for pollen as a source of food from one flower to another. The pollen grains that are deposited on its head and hind legs are the promoting source of cross pollination. The butterflies made 3-4 visits/ flower with each visit of 10-15 seconds. They inserted their proboscis in to corolla tube and collect nectar. These are nectar robbers. The insects like ants (*Paratrichina* sp. & *O. smaragdina*) and *Drosophila melanogaster*, visiting the flowers were merely visitors or nectar robbers. On the basis of visitation rates, pollen load on their body parts and transfer of pollen load on virgin stigma it was found that *Nectarinia asiatica*, *Nectarinia zeylonica and Tetragonula irridipennis* are most efficient pollinators. No pollen was deposited on any of the Vaseline coated slides hung on the branches; hence, it was concluded that wind has no role in the pollination of this species.

Figure 1 *Etlingera elatior*. (a) Inflorescence. (b) Pollinated single flower. (c) *Tetragonula irridepennis* pollinating flower (d) Microscopic view of *Tetragonula irridepennis*, Scale bar is 1mm (e) Infructescence. (f) Stamen. (g) Stigma. (h) Pollen vibility – Tetrazolium chloride test (V- Viable, NV- Non viable). (i) Pollen tube in Brewbacker and Kwack's medum. (j, k) Birds Pollinating flower (j) *Nectarinia zeylonica* (Female). (k) *N. zeylonica* (Male).

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, confirmed the absence of autogamy in the species. Bagged flowers was pollinated by pollen from another flower of the same plant resulted 8 % fruit set and pollinated with pollen from another plant resulted 24 % fruit set.

Pollination efficiency

To understand the possible reasons for poor fruit set under open pollinations, pollination efficiency was investigated by screening a large number of stigmas at the evening under the microscope for the presence of pollen. Of the 100 flowers examined from population A, pollen grains present only on stigmas of 30 % of the remaining 70 % of the stigmas did not have pollen grains. Pollination efficiency of birds (*N. asiatica, N. zeylonica*) and *Tetragonula irridipennis* indicate that these are the major and effective pollinators of *E. elatior*.

Fruit set and seed set

Fruit set and seed set are varied considerably from one inflorescence to other inflorescence. Fruit development and maturation are prolonged and take 2–3 months (Fig. 1e). The percentage of fruit set under manual cross pollination (Xenogamy) was higher than open/natural pollination. The percentage of fruit set under manual self pollination was very poor (8 %).

DISCUSSION

The present work provides the first detailed study of the reproductive biology of *E.elatior*. Phenology involves the study of flower initiation, development, anthesis, fruiting *etc.* It is related to season and climatic factors. Therefore detailed information of phenology is important for the study of reproductive biology. The data presented in this study indicate that the flowering phenology of *E.elatior* is all year round, with a peak flowering January to April. During the peak flowering period 18 – 24 flowers bloom on each inflorescence. One inflorescence of *E.elatior* is long and last for about 3 – 4 months. Anthesis occurs between 5.30 – 6.30 a.m. and the lifespan of single flower was 1 day. Similar observations were found in other genera of Zingiberaceae, previously reported by various authors [7, 8, 9, 10, 11, 12, 2, 13, 14, 15, 16].The flowers are bright red with yellow margin in colour [17].

The amount of reward offered to a flower visitor also depends on the concentration of sugar in the nectar. Nectar secretion starts soon after anthesis and accumulates gradually and reaches about 47.6±1.40 µl by 9.00 a.m. (highest volume of nectar). Then the volume gradually decline to 27.1±1.45 µl by on 6.00 p.m. Difference in temperature and humidity could account for the variation seen in the total nectar volumes. [18] cited that humidity strongly influences nectar secretion. [19] made a point in citing that variation in temperature, humidity and soil moisture can influence high rates of nectar production. These factors have a direct influence on the plant metabolism, which can cause them to be important determinants of nectar secretion rates [20]. These studies maintain that an increase in these environmental factors can increase nectar productivity, however it is [21] that cited an inverse correlation between nectar sugar content and relative humidity. He showed that even though the actual volume of nectar produced may increase; sugar concentration does not necessarily increase. The highest sugar concentration (26.02±1.51%) occurs at 9.00 a.m., and there after the concentration also declined, and reached to 22.05±1.43 % by 6.00 p.m.

On the basis of visitation rates, pollen load on their body parts and transfer of pollen load on virgin stigma, it was found that *Nectarinia asiatica*, *Nectarinia zeylonica*, *Tetragonula irridipennis*, *Certina* sp. are most efficient pollinators. [17] also suggested that *E. elatior* may be bird pollinated. *Etlingera brevilabris* and *Hornstedtia tomentosa* have *Arachnothera* spp. (Nectariniidae) as the pollinator. The latter species of Zingiberaceae both have red flowers and basal inflorescences. Three species of *Etlingera* and five other species of Alpinieae, were pollinated by spiderhunters, while 12 species (two *Costus* spp., *Globba brachyanthera*, *Zingiber longipedunculatum* and the rest of Alpinieae) were pollinated by *Amegilla* bees. Halictid bees were found to be pollinators of three species of *Boesenbergia* and seven species of Alpinieae [22]. However, spiderhunters were found to be pollinators of *E. elatior* in a different area in Khao Nan National Park, about 17 km away from the study site. They are also reported to be pollinators for three species of *Etlingera*, *i.e. E.* aff. *metriocheilos*, *E. punicea* and *E.* aff. *brevilaris* in Borneo [22]. In addition, ants are found to be nectar thieves. The ants cover the whole inflorescence with soil, making it impossible for other pollinators to reach the flower's reproductive organs [23]. The results based on hanging Vaseline coated slides indicate that *E. elatior* is not an anemophyllous plant. No reports on the wind pollinated plants of Zingiberaceae had been published.

The present work suggests that *E. elatior* is xenogamous and bird or insect pollinated. Percentage of fruit set in artificial cross pollination was higher than that resulting from natural pollination, which strongly indicates that some external agents are required for effective pollination.

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