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Floral biology and pollination behaviour of *Gloriosa rothschildiana*

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Abstract
The flowers of *Gloriosa rothschildiana* were borne on a short pedicel (7.73 cm) and were solitary. The flower size was small (1.60 g). There were six small crimson colored tepals (3.60 x 1.45 cm) with short stamen (3.34 cm) and pistil (3.39 cm). The stamen displayed profuse orange-yellow pollen. The pistil possessed three celled ovary which formed an ellipsoidal capsule. The mean number of days taken for completion of flowering phases was 20.70 days for *G. rothschildiana*. The percentage of bud opening and anther dehiscence in *G. rothschildiana* was maximum at 9.30 am. The percentage of stigma receptivity, pollen viability and fertility were maximum on the day of anthesis. Pollens were oval shaped and the pollen output was minimum in *G. rothschildiana.* The highest pod set was observed under artificial cross pollination followed by self-pollination and natural open pollination.

Keywords: Floral biology, pollination, *Gloriosa rothschildiana*

Introduction
Gloriosa rothschildiana L., a climber belonging to the family Colchicaceae is a major high value medicinal crop. Seeds and tubers of *Gloriosa* species contain valuable alkaloids *viz*., colchicine and colchicoside as the major constituents, which are used to treat gout and rheumatism. Due to the action of colchicine on spindle fibre formation during cell division, the plant has been identified as a potential anti-cancerous drug. The flowers are large, solitary or may form a lax-corymbose inflorescence, twisted and crisped with six recurved or reflexed petals, blossoming yellow but changing to yellow-red and deep scarlet.
Though some information regarding the floral biology have been recorded by the early Gloriosa workers like Narain (1976) [7], Mamatha (1989) [4], Rajagopalan (1994) [8] and Gupta and Raina (2001) [3], Nagajothi (2008) [6], Rajamani *et al.* (2009) [9]. No systemic studies have been undertaken withdefinite and precise research approaches. Information on pollination biology not only required for comprehensive understanding of breeding system of a species and its evolutionary success but also for effective optimization of yield, conservation and rational genetic improvement. Keeping the above facts in view a detailed study of the reproductive biology of *Gloriosa rothschildiana* was undertaken at Tamil Nadu Agricultural University, Coimbatore, India.

Materials and methods
Tubers of *G. rothschildiana* were planted in the furrows of at the distance of 20 cm apart and covered with top soil. The selected plants were labeled forthe convenience of observing the characters included in the study of reproductive biology. Observations were made on floral morphological characters *viz.*, pedicel length (cm), tepal length and breadth (cm), length of the stamen and pistil (cm) and flower weight (g).
Ten flowers of *G. rothschildiana* were tagged at the time of appearance of flower bud for tracing the number of days taken for completion of different flowering phases. A series of developmental stages in the *Gloriosa* were categorized as bud initiation, bud opening, pre anthesis, anthesis, post pollination stage Farooqi and Sreeramu (2004) [1], Singh (2006) [11]. Observations on bud opening were recorded during peak flowering season. The observation were also recorded on anther dehiscence, sigma receptivity, pollen viability and fertility. Breeding behaviour, pollination efficiency and extent of pod set was studied by employing different modes of pollination *viz.*, natural open pollination, self pollination and artificial cross pollination as adopted by Mamatha *et al.* (1993) [5].

Results
The flowers are large, axillary and solitary, with pedicels which are reflexed near tip. They are incomplete, ebracteolate, perfect, regular, hypogynous and acropetal. Flowers contain

nectariferous structures inviting bees, butterflies and small insects. Petaloid, persistant, tepal six with strongly crinkled wavy margin, narrow and linear in shape, reflexed, greenish at first, then yellow, passing through orange and crimson. They are arranged in valvate and induplicate aestivation. The peculiar structure of the small flowers with six perianth lobes bent backwards, six radiating anthers and the style bent almost 90° at the point of attachment to the ovary does not make them suitable for pollination by small insects. Stamens comprise six, hypogynous anther, linear, dorsifixed, versatile and dehisce extrorsely to shed bright yellow pollen in abundance. Ovary is superior, tricarpellary, syncarpous, monolocular, numerous ovules on parietal placenta, style sharply deflexed at a right angle from the ovarian axis, stigma trifid. Fruit is a loculicidal, oblong capsule 4-6 cm x 1-2 cm, containing a fleshy, red sarcotesta.

Flower morphology (Table 1)

The individual flower weighed 2.52 g. *G. rothschildiana* flowers were borne on a short pedicel (7.73 cm) and are solitary. The flower size was small (1.60 g) with six small crimson colored tepals (3.60 x 1.45 cm) bearing short stamen (3.34 cm) and pistil (3.39 cm). (Plate 1, 2, 3).

Table 1: Flower morphology of *Gloriosa rothschildiana*

Characters	Dimensions
Pedicel length	7.73 (cm)
Tepal size	3.60 X1.45 (cm)
Length of stamen	3.34 (cm)
Length of the pistil	3.39 (cm)
Flower weight	1.60 (g)
Avg. pollen size	65.68 (µm)
Pollen output (Nos.)	7,01,250
Pollen germination %	98.08 %
Pollen fertility %	98.33 %
Natural open pollination	76.66 % of pod set
Self pollination	86.66 % of pod set
Artificial cross pollination within the species	93.00 % of pod set

Floral biology

The flowering phase ranged from 15 to 24 days (mean of 20.70 days). The maximum mean percentage of flower bud opening was observed between 8.30 to 9.30 am (60.00 per cent) followed by 7.30 to 8.30 am (27.00 per cent). The microscopical observation revealed the extrose, longitudinal splitting of anthers in *G. rothschildiana*. The colour of the anther lobe was creamy yellow bearing orangish yellow pollen grains in both the species. With respect to the time of anther dehiscence, it started from 6.30 am and continued upto 10.30 am. More anthers dehisced between 8.30-9.30 am and the percentage of dehiscence occurring during this period was 54.00 per cent. The anthers dehisced between 7.30-8.30 am was 29.00 per cent.

Stigma receptivity (Fig 2)

The results showed that the maximum stigma receptivity was observed on the day of anthesis (97.50 per cent of pod set). But, on the day of anthesis, maximum stigma receptivity was from 8.00 am to 11.00 am in *G. rothschildiana*, indicating that the stigma receptivity was maximum on the day of anthesis.

Pollen morphology, viability and fertility

Based on the appearance of exine of the pollen grain, the shape of the pollen was oval in all the pollen samples observed. The mean germination percentage and pollen tube growth was maximum on the day of dehiscence which recorded 98.08 per cent and 47.78 µm respectively. The percentage of fertile pollen was 98.33 per cent on the day of dehiscence and gradually decreased to 91.17 per cent on the third day.

Pollen size and pollen output

The average pollen production per flower was 7, 01, 250 with average size of 65.68 µm.

Pollination methods for pod set

G. rothschildiana recorded 93.00 per cent pod set under artificial cross pollination, 86.66 per cent under self-pollination and 76.66 per cent under natural open pollination.

Table 2: Pollen viability of *Gloriosa rothschildiana*

Age of pollen grain (day)			Pollen fertility %	Sterile pollen %
On the day of dehiscence (1st day)	I	98.08	98.33	1.67
	II	47.72		
Second day	I	89.20	95.19	4.81
	II	43.61		
Third day	I	88.65	91.17	8.83
	II	42.96		

I - Germination percentage (%)

II - Maximum length of pollen tube (µm) after 2 hours of dating

Discussion

The study of floral biology *viz*., flower morphology, pollination behavior, and barriers in pollination of any crop is very important for crop improvement. Glory lily is a cross pollinated species and these fundamental information including anthesis, stigma receptivity, pollen viability and fertility *etc.*, are much needed for programming crop improvement through hybridization.

In the present investigation on flower morphology of *G. rothschildiana*, the flowers were small, with crimson colored tepals bearing short stamen and pistil. These descriptions are in accordance with the reports of Gupta and Raina (2001) [3], Farooqi and Sreeramu (2004) [1], Singh (2006) [11], and Rajamani *et al.* (2009) [9].

In the present study, the number of days taken for completion of flowering phases in *G. superba* and *G. rothschildiana* was recorded right from the date of bud initiation to the date of pod set. There were five stages of flower development *viz*., bud initiation, bud opening, pre-anthesis, anthesis, post pollination stage. In all these stages, the flower colour changed pertaining to each stage of flower development.

The bud opening stage in *G. rothschildiana* was characterized by light green colour. This was followed by the pre-anthesis stage when the perianth turned yellow with crimson in the middle. Post pollination was characterized by the upper half of the perianth lobes being crimson coloured and the lower portion being yellow coloured. Lastly, the perianth lobes turned entirely into crimson coloured. Singh (2006) [11] also made similar reports about the different stages of flowering in *G. rothschildiana*.

In general, the duration of flowering phase was 20.7 days in *G. rothschildiana*. Mamatha (1989) [4] and Rajamani *et al.* (2009) [9] also opined that the period of flower bud development extended upto 17 to 20 days depending upon the season.

In *Gloriosa*, the flowers bloomed during morning hours after the onset of sun. This speaks on to the magnitude role of sunlight in triggering the flower opening process and appears to be the nature's provision for ensuring pollination. In the present investigation, the bud opening in started from 6.30 to 7.30 am and increased gradually after reaching the peak at 9.30 am and there after started declining and reached the minimum between 9.30 to 10.30 am, beyond which no flowers opened. This is in agreement with that of Mamatha (1989) [4] and Rajamani *et al*. (2009) [9], who stated that the peak period of bud opening in *Gloriosa species* was between 8.30 to 10.30 am.

In the present study, the anther dehiscence in started from 6.30 am and reached the peak at 9.30 am and there after started declining and reached the minimum at 10. 30 am. This indicated that glory lily is photosensitive and anthesis corresponded to the sunlight falling on the plants. Thereupon (after 10.30 am), as the intensity of sunlight is more, the anthesis slowed down. Narain (1976) [7], Mamatha (1989) [4], Rajagopalan (1994) [8], Nagajothy (2008) and Rajamani *et al.* (2009) [9] also reported similar observation on anthesis in *G. superba*.

In the present study, the stigma receptivity was assessed by carrying out artificial pollination of flowers under controlled conditions. The pod set of 97.50 per cent was observed in flowers which were pollinated on the day of anthesis, indicating the maximum receptiveness of stigma during anthesis. The flowers pollinated one day before anthesis exhibited the lowest mean percentage of pod set indicating that the stigma was premature or not ready for receptivity during that period. In general, the percentage of pod set was higher in the early morning hours (7.00 to 11.00 am) irrespective of the pollination done on different days.

In general, the stigma remains receptive for three days *viz*., one day prior to anthesis, on the day of anthesis, one day after anthesis. These receptive periods coincided with pre-anthesis, anthesis and post pollination stage of flower development. The loss of stigma receptivity can be identified from the change in stigma colour from green to red in both the species. Varying reports were made by Rajagopalan (1994) [8] and Gupta and Raina (2001) [3] who found that the stigma was receptive for about three days after anthesis. This may be due to variation in environmental conditions such as temperature, humidity or dew, rainfall and season.

Based on the appearance of exine of the pollen grains, oval or elliptical shaped pollen were observed and similar findings were made by Mamatha *et al*. (1993) [5] who reported the presence of oval shaped pollen with smooth exine. Ravikumar and Nair (1986) [10] also reported the presence of ellipsoidal pollen in the interspecific tetraploid hybrids of *Gloriosa*.

In the present investigation, the average pollen size was 65.24 µm in *G. rothschildiana*. These observations are in accordance with the findings of Mamatha *et al*. (1993) [5]. The pollen output was lower in *G. rothschildiana* when compared to the other species of *Gloriosa.* This was due to the smaller pollen sac it possesses.

The high pollen fertility observed in the present investigation is in conformity with the observation by Narain (1976) [7] in *G. superba*. Mamatha *et al*. (1993) [5] also observed in *G. superba* that 50 per cent of pollen was viable even six hours after dehiscence.

Pollen viability is an ability of a pollen grain to germinate and develop as a pollen tube (Gerard, 1932) [2]. The growth of the pollen tube can be taken as the measure of pollen viability since the non-viable pollen could not make the growth of a pollen tube. Good pod set cannot be achieved unless pollen is viable with high germination percentage.

The pollen germination percentage and mean length of pollen tube was higher on the day of anther dehiscence and a gradual reduction was observed thereafter as the age of the pollen grains advanced. This is normally expected since aged pollen grains might have lesser moisture content, leading to the deterioration of viability. This is in agreement with the findings of Mamatha *et al*. (1993) [5] who reported that 98.20 per cent of pollen germination was observed in 10 per cent sucrose concentration.

In the present study on pod set under different pollination methods in *G. rothschildiana*, maximum pod set was observed in artificial cross pollination within the species followed by self-pollination. Minimum pod set was noticed in natural self-pollination. This is due to typical flower shape during the flower development. The peculiar structure of the large flowers with six perianth lobes bend backwards, six radiating anthers and the style bend almost 90º at the point of attachment to the ovary, does not make them suitable for pollination by small insects. These findings are in accordance with the experiments of Narain (1976) [7], Rajagopalan (1994) [8] and Sudhendra and RudreGowda (1997) [12]. Low seed set under natural pollination was also observed by Gupta and Raina (2001) [3] in *G. superba.*

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