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Reproductive Biology of *Gloriosa rothschildiana*

Anandhi Selvarasu* • **Rajamani Kandhasamy**

Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India

Corresponding author: * **sanandhijasmin@gmail.com**

ABSTRACT

An investigation was carried out to study the reproductive biology of *Gloriosa rothschildiana*. The flowers were borne on a short pedicel (7.73 cm) and were solitary. The flower weighs 1.60 g. There were six small crimson-colored tepals (3.60 × 1.45 cm) with a short stamen (3.34 cm) and pistil (3.39 cm). The stamen displayed profuse orange-yellow pollen. The pistil possessed a three-celled ovary which formed an ellipsoidal capsule. The mean number of days taken to complete flowering was 20.70 days. The percentage of bud opening and anther dehiscence in *G. rothschildiana* was 60% at 9.30 am. The maximum percentage of stigma receptivity (97.5%), pollen viability (98.1%) and fertility (98.33%) were observed on the day of anthesis. Pollen was oval shaped and pollen output was 701,250 in *G. rothschildiana.* The highest pod set (93%) was observed under artificial cross pollination followed by self-pollination and natural open pollination.

Keywords: Glory lily, flower, morphology, palynology

INTRODUCTION

Gloriosa rothschildiana L., a climber belonging to the Colchicaceae family, is a major high value medicinal crop. In Indian market, the annual trade estimated is 100-200 million tonnes and the price range is Rs. 600-750/kg (Ved 2007). The plant is known as glory lily, creeping lily or flame lily in English, *kazhappaikizhangu*, *kanvalikizhangu*, *karthigaikizhangu* or *sengandhal* malar in Tamil, *kalihari* in Hindi, *tangiballi* in Kannada, *manthorikhizangu* in Malayalam and *kalappagadda* in Telugu (Sundar *et al*. 2006). *Gloriosa* occurs naturally in Africa and Southeastern Asia. In India, it is usually found in Himalayan foot-hills, central India, Tamil Nadu, Andhra Pradesh and Bengal.

The important species found in India are *G. superba* and *G. rothschildiana* (Farooqi and Sreeramu 2004). 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 (Rajamani *et al*. 2009).

The plant grows in sandy-loam soil in the mixed deciduous forests in sunny positions. It is extremely tolerant to nutrient-poor soils. It occurs in thickets, forest edges and boundaries of cultivated areas in warm countries up to 2530 masl (Neuwinger 1994; Inchem 2004).

The vines grow tall (3.5 to 6 m), very thin, and are weak stemmed with 'V' shaped tuberous roots. The vines have cirrhosed leaf tips which cling to the support. The leaves are ovate, lanceolate, accuminate, tips spirally twisted to serve as tendrils. 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 (Farooqi and Sreeramu 2004; Rajamani *et al*. 2009).

Though some information regarding the floral biology has been recorded by early *Gloriosa* researchers (Pollination mechanism - Narain (1976); Palynology, pollination mechanism - Mamatha (1989); Stigma receptivity – Rajagopalan (1994); Sequential flowering habit - Gupta and Raina (2001); Palynology - Nagajothi (2008)), no systemic studies have been undertaken with definite 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 *G. rothschildiana* is presented.

MATERIALS AND METHODS

The study on reproductive biology was carried out at the Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during August-January, 2011 in *G. rothschildiana.* Tubers of *G. rothschildiana* were collected from Mulanur farmers, Tirupur district, Tamil Nadu.

Tubers were planted in the furrows of at the distance of 20 cm apart and covered with top soil. The selected plants were labeled for the 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; Singh 2006).

Observations on bud opening were recorded during peak flowering season. The number of buds opened (stage C of flower development) was recorded for ten days at one hour interval, right from 6.30 to 10.30 am. After recording, the counted flowers were tagged to avoid duplication and the per cent of bud opened at different time interval was recorded. The time at which maximum per cent of bud opened was recorded as the time of bud opening.

Ten flowers at pre-anthesis stage were tagged for 10 days before anther dehiscence in both the species. Observations for de-

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Research Note

hiscence were recorded on next day *i.e*., the anthesis stage at one hour interval from 6.30 to 10.30 am. Appearance of longitudinal split in the pollen sac indicated the commencement of anther dehiscence. The per cent of anther dehiscence at different time interval was worked out and the time at which maximum per cent of anther dehisced was recorded as the time of anther dehiscence.

To assess the duration of the stigma receptivity, artificial pollination of flowers was carried out under controlled condition during pre-anthesis, anthesis and one day after anthesis. Flowers were emasculated at pre-anthesis stage and covered with butter paper cover. Ten flowers were pollinated at the above mentioned three stages starting from 7.00 am to 11.00 am at one hour interval. Pollen grains were dusted over the receptive stigma. Flowers were bagged with paper cover after pollination. The bags were removed 10 days after pollination and those that showed pod set were counted to assess the receptivity of stigma.

Fresh pollen collected from five flowers was used for observing the shape of the pollen under compound microscope. Based on the visual appearance, the shapes were scored as round or oval. Pollen output was estimated using a haemocytometer (Model-RSS-151, R.S Scientific, India) as suggested by Sathiamoorthy (1973). The pollen viability and fertility were studied by acetocarmine (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and *in vitro* pollen germination tests Sathiamoorthy (1973)

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). Each method of pollination was imposed in 30 flowers.

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 (**Fig. 1C, 1D, 1E**). 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 (**Fig. 1H, 1I**). Fruit is a loculicidal, oblong capsule 4-6 cm × 1-2 cm, containing a fleshy, red sarcotesta.

Flower morphology

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 × 1.45 cm) bearing short stamen (3.34 cm) and pistil (3.39 cm) (**Table 1**).

Days for completion of flowering phase

The flowering phase ranged from 15 to 24 days (mean of 20.70 days) (**Table 2**).

Time of flower bud opening

The maximum mean percentage of bud opening was observed between 8.30 to 9.30 am (60.00%) followed by 7.30 to 8.30 am (27.00%) (**Fig. 1A, 1B; Table 3**).

Time of anther dehiscence

The microscopical observation revealed the extrose, longitudinal splitting in *G. rothschildiana*. The colour of the anther lobe was creamy yellow bearing orangish yellow pollen grains (**Fig. 1F**). With respect to the time of anther dehiscence, it started from 6.30 am and continued up to 10.30 am. More anthers dehisced between 8.30-9.30 am and the percentage of dehiscence occurring during this period was 54%. The anthers dehisced between 7.30-8.30 am was 29% (**Table 4**).

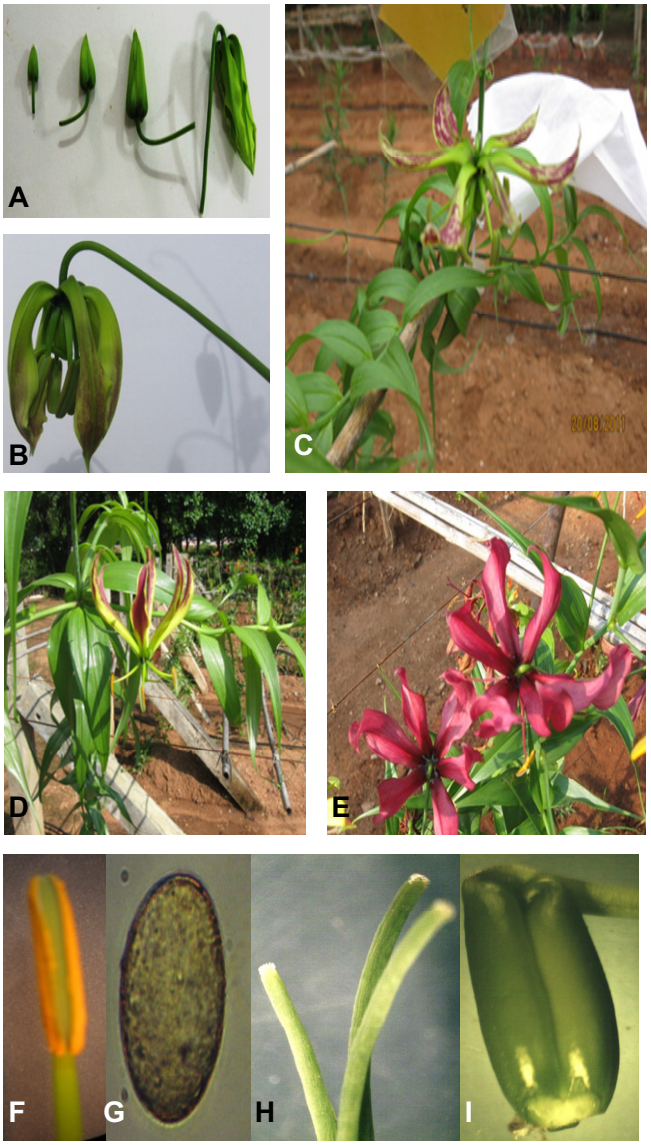

Fig. 1 Floral biology of *G. rothschildiana*. (**A**) Bud development, (**B**) bud opening, (**C**) pre-anthesis, (**D**) anthesis, (**E**) post pollination stage, (**F**) microscopic view of anther, (**G**) pollen, (**H**) trifid stigma and (**I**) ovary.

Table 1 Flower morphology of *Gloriosa rothschildiana*.

Characters	Dimensions
Pedicel length	7.73 (cm)
Tepal size	3.60 × 1.45 (cm)
Length of stamen	3.34 (cm)
Length of the pistil	3.39 (cm)
Flower weight	1.60 (g)
Average pollen size	65.68 (µm)
Pollen output (No.)	7,01,250
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

Stigma receptivity

The results showed that the maximum stigma receptivity was observed on the day of anthesis (97.50% of pod set).

Table 2 Number of days taken for completion of flowering phase in *Gloriosa rothschildiana*.

Flower no.	Date of different flowering phase						Duration of flowering phase (days)
	A	B	C	D	E	F	
1	6.9.11	15.9.11	17.9.11	19.9.11	19.9.11	25.9.11	20.00
2	7.9.11	19.9.11	22.9.11	23.9.11	24.9.11	29.9.11	23.00
3	8.9.11	21.9.11	22.9.11	23.9.11	23.9.11	27.9.11	20.00
4	9.9.11	20.9.11	21.9.11	22.9.1	23.9.11	29.9.11	21.00
5	10.9.11	21.9.11	22.9.11	23.9.11	24.9.11	1.10.11	21.00
6	11.9.11	23.9.11	24.9.11	26.9.11	27.9.11	3.10.11	23.00
7	12.9.11	25.9.11	26.9.1	28.9.11	30.9.11	5.10.11	24.00
8	13.9.11	20.9.11	22.9.11	23.9.11	24.9.11	27.9.11	15.00
9	14.9.11	28.9.11	28.9.11	29.9.11	29.9.11	3.10.11	20.00
10	15.9.11	26.9.11	27.9.11	29.9.11	29.9.11	4.10.11	20.00
Mean							20.70

A: Bud initiation; B: Bud opening; C: Pre-anthesis; D: Anthesis; E: Post pollination stage

Table 3 Time of bud opening in *Gloriosa rothschildiana*.

Date	Flowers that opened at different hours (%)			
	6.30-7.30 am	7.30-8.30 am	8.30-9.30 am	9.30-10.30 am
16.8.11	10	30	50	10
17.8.11	0	20	80	0
18.8.11	20	20	60	0
19.8.11	10	30	50	10
20.8.11	0	40	60	0
21.8.11	20	20	60	0
22.8.11	10	20	60	10
23.8.11	10	30	60	0
24.8.11	0	40	50	10
25.8.11	0	20	70	10
Mean	8	27	60	5

Table 4 Time of anther dehiscence in *Gloriosa rothschildiana*.

Date	No. of flowers observed/day	Anther dehiscence at different hours (%)			
		6.30-7.30 am	7.30-8.30 am	8.30-9.30 am	9.30-10.30 am
27.9.11	10	20	30	50	0
1.10.11	10	20	20	60	0
3.10.11	10	10	40	40	10
9.10.11	10	20	10	70	0
15.10.11	10	10	30	40	20
16.10.11	10	0	10	80	10
19.10.11	10	0	50	40	10
22.10.11	10	20	20	60	0
25.10.11	10	0	20	70	10
28.10.11	10	0	60	30	10
Mean		10	29	54	8

Table 5 Stigma receptivity of *Gloriosa rothschildiana*.

Time of pollination	Hours (am)	No. of flowers pollinated	No. of pod set	% of pod set	Mean
One day before anthesis	7.00-8.00	10	9	90	92.50
	8.00-9.00	10	9	90	
	9.00-10.00	10	9	90	
	10.00-11.00	10	10	100	
On the day of anthesis	7.00-8.00	10	9	90	97.50
	8.00-9.00	10	10	100	
	9.00-10.00	10	10	100	
	10.00-11.00	10	10	100	
One day after anthesis	7.00-8.00	10	9	90	95.00
	8.00-9.00	10	9	90	
	9.00-10.00	10	10	100	
	10.00-11.00	10	10	100	

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 (**Table 5**).

Pollen morphology

The fresh pollen collected from five flowers of *G. rothschildiana* was observed under the compound microscope. Based on the appearance of exine of the pollen grain, the shape of the pollen was oval in all the pollen samples observed (**Fig. 1F, 1G**).

Pollen viability and fertility

The mean germination percentage and pollen tube growth was maximum on the day of dehiscence which recorded 98.08% and 47.78 µm, respectively. The percentage of fertile pollen was 98.33% on the day of dehiscence and gradually decreased to 91.17% on the third day (**Tables 6, 7**).

Pollen size and pollen output

The average pollen production per flower was 701,250 with average size of 65.68 µm.

Pollination methods for pod set

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

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 col-

Table 6 Pollen viability of *Gloriosa rothschildiana*.

Age of pollen grain (day)		Source 1	Source 2	Source 3	Source 4	Source 5	Mean
On the day of dehiscence (1st day)	I	96.00	98.41	98.00	100.00	98.00	98.08
	II	47.26	48.41	49.07	47.37	46.51	47.72
Second day	I	92.00	84.00	86.00	88.00	96.00	89.20
	II	44.67	48.74	40.58	42.11	41.95	43.61
Third day	I	87.69	93.54	90.56	91.48	80.00	88.65
	II	42.89	43.63	41.61	41.95	44.75	42.96

I - Germination percentage (%)
II - Maximum length of pollen tube (µm) after 2 h of dating

Table 7 Pollen fertility in *G. rothschildiana*.

Age of the pollen grains	No. of pollen grains tested	Fertile pollen (%)	Sterile pollen (%)
First day of dehiscence	100	97.00	3.00
	100	98.00	2.00
	100	100.00	0.00
	Mean	98.33	1.67
Second day	100	94.00	6.00
	100	96.00	4.00
	100	95.58	4.42
	Mean	95.19	4.81
Third day	100	88.57	11.43
	100	93.54	6.46
	100	91.42	8.58
	Mean	91.17	8.83

ored tepals bearing short stamen and pistil. These descriptions are in accordance with the reports of Gupta and Raina (2001), Farooqi and Sreeramu (2004), Singh (2006), and Rajamani *et al.* (2009).

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) 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) and Rajamani *et al*. (2009) 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) and Rajamani *et al*. (2009), who stated that the peak period of bud opening in *Gloriosa* spp. 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), Mamatha (1989), Rajagopalan (1994), Nagajothy (2008) and Rajamani *et al.* (2009) 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% 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 species. Varying reports were made by Rajagopalan (1994) and Gupta and Raina (2001) 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) who reported the presence of oval shaped pollen with smooth exine. Ravikumar and Nair (1986) 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). 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) in *G. superba*. Mamatha *et al*. (1993) also observed in *G. superba* that 50% of pollen was viable even 6 h after dehiscence.

Pollen viability is an ability of a pollen grain to germinate and develop as a pollen tube (Gerard 1932). 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) who reported that 98.20% of pollen germination was observed in 10% sucrose.

In the present study on pod set under different pollina-

tion 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 90^{o} 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), Rajagopalan (1994) and Sudhendra and RudreGowda (1997). Low seed set under natural pollination was also observed by Gupta and Raina (2001) in *G. superba*.

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