Reproductive biology of *Gloriosa superba*

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Abstract:

An investigation was carried out to study the reproductive biology of *Gloriosa superba* at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2011 - 2012. The flowers of *Gloriosa superba* were born on a long pedicel (10.45 cm). The scarlet red coloured tepal was 6.80 x 1.31 cm in dimension. The six radiating anthers were 3.34 cm long and the style was 6.40 cm. The individual flower weighed 2.52 g. The stamen displayed profuse orange-yellow pollen. The pistil possessed three loculed ovary which formed an ellipsoidal capsule. Mean number of days taken for completion of flowering phases was found to be 21.10 days and the percentage of bud opening and anther dehiscence was maximum at 9.30 am. The percentages of stigma receptivity, pollen viability and fertility were maximum on the day of anthesis. Pollens were oval shaped. The highest pod set was observed under artificial cross pollination followed by self-pollination and natural open pollination.

Key words: Glory lily, anthesis, stigma receptivity, pollination, pollen morphology

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Introduction

*Gloriosa superba* L., a climber belonging to family Colchicaceae is a major high value medicinal crop cultivated in Tamil Nadu. *Gloriosa* derives its name from the word ‘gloriosus’, which means handsome and *superba* from the word ‘superb’ means splendid or majestic kind. The plant is known as glory lily, creeping lily or flame lily in English, Kazhappakizhangu, Kanvalikizhangu, Karthugaikizhangu or Sengandhal malar in Tamil, Kalihari in Hindi, Tingiballi in Kannada, Manthorikhizangu in Malayalam and Kalappagadda in Telugu (Sundar et al., 2006). *G. superba* occurs naturally in Africa and Southeastern Asia. In India, it is usually found in Himalayan foothills, Central India, Tamil Nadu, Andhra Pradesh and Bengal. 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 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 MSL (Mean Sea Level in meters) (Neuwinger, 1994; Inchem, 2004).

The vines grow tall, 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, acuminate, 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. The peculiar structure of the large 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.

Though some information regarding the floral biology have been recorded by the early Gloriosa workers like Narain (1976), Mamatha (1989), Rajagopalan (1994) and Gupta & Raina (2001), no systematic 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 *Gloriosa superba* was undertaken at Tamil Nadu Agricultural University, Coimbatore, India.

Materials and methods

The studies on floral and pollination biology were 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. superba*. Tubers of *Gloriosa superba* were collected from Mulanur, Tirupur district, Tamil Nadu, where it was grown commercially by the farmers in a larger extent.
Tubers were planted in the furrows of at a distance of 20 cm apart and covered with top soil. The selected plants were labeled for convenience of observing the characters including study of floral 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. superba were tagged at the time of appearance of flower bud for tracing the number of days taken for completion of different flowering phases. Series of developmental stages in the Gloriosa were categorized as bud initiation, bud opening, pre anthesis, anthesis, post pollination stage (Farooqi & Sreeramu, 2004; Singh, 2006).

Observations on bud opening were recorded during peak flowering season. The number of buds opened (stage C of flower development) per day 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 and the time at which maximum per cent of bud opened was noted from the data.

Ten flowers at pre-anthesis stage were tagged before anther dehiscence for ten days. Observations for dehiscence 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.

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 bag. 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 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 made by Haemocytometer method as suggested by Sathiamoorthy (1973). The pollen viability was studied by acetocarmine and in vitro pollen germination tests (Sathiamoorthy, 1973).

Breeding behaviour, pollination efficiency and extent of pod set were studied by employing different modes of pollination viz., natural open pollination, self pollination and artificial cross pollination as adopted by Mamatha et al. (1993).

Results

The flowers are large, axillary and solitary, with pedicels which are reflexed near tip. They are incomplete, ebracteolate, perfect, regular, hypogynous and acroptal. Flowers contain nectariferous structures inviting bees, butterflies and small insects. Petaloid, persistant, tepals are six with strongly crinkled wavy margin, narrow and linear in shape, reflexed, greenish at first, then yellow, passing through orange and scarlet to crimson. They are arranged in valvate and induplicate aestivation. The peculiar structure of the large 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 anthers, linear, dorsifixed, versatile and dehisce extrorsely to shed bright yellow pollen in abundance. Ovary is superior, tricarpellary, syncarpous, monolocular with numerous ovules on parietal placentation, style sharply deflexed at a right angle from the ovarian axis, stigma trifid. Fruit is a loculicidal, oblong capsule of 4-6 cm x 1-2 cm, containing a fleshy, red sarcotesta.

Flower morphology

The flowers of G. superba were born on the long pedicel (10.45 cm). The scarlet red coloured tepal was 6.80 x 1.31 cm in dimension. The six radiating anthers were 3.34 cm long and the style was of 6.40 cm. The stamen displayed profuse orange- yellow pollen. The pistil possessed three loculed ovary which formed an ellipsoidal capsule. The individual flower weighed 2.52 g (Table 1).

Days for completion of flowering phase

The flowering phase in G. superba extended from 18 to 23 days (mean of 21.10 days) (Table 2)

Time of flower bud opening

In G. superba, maximum mean percentage of flower bud opening in a day was observed between 8.30 to 9.30 am (52.00 per cent) followed by 7.30 to 8.30 am (30.00 per cent).

Time of anther dehiscence

The microscopical observation revealed the extrose, longitudinal anther splitting in G. superba. The colour of the anther lobe was creamy yellow bearing orangish yellow pollen grains.

Anther dehiscence in a day started from 6.30 am and continued up to 10.30 am (Fig.1). More anthers...
dehisced between 8.30-9.30 am and the percentage of dehiscence occurring during this period was 50.00 per cent. The anthers dehisced between 7.30-8.30 am in G. superba was 33.00 per cent.

**Stigma receptivity**

The results showed that maximum stigma receptivity was observed on the day of anthesis (97.50 per cent of pod set) in G. superba. But, on the day of anthesis, maximum stigma receptivity was from 7.00 am to 10.00 am (Fig.2).

**Pollen morphology**

The fresh pollen collected from five flowers of G. superba was observed under the compound microscope and shape of the pollen was found as oval.

**Pollen**

In G. superba, mean germination percentage and pollen tube growth after two hours of incubation was maximum (96.20 per cent and 43.81 μm) on the day of dehiscence followed by the second day and third. The percentage of fertile pollen in G. superba was maximum (98.01 per cent) on the first day of dehiscence followed by second day (91.66 per cent) and third day (86.66 per cent) (Fig 3)

**Pollen size and pollen quantification**

Average pollen production per flower in G. superba was 7,30,000, with the average size of 52.82 μm.

**Pollination methods for pod set**

The pod set for the various pollination methods viz., natural open pollination, self-pollination and artificial cross pollination in G. superba was studied (Table 3). The data indicated that in G. superba, the highest pod set was observed in artificial cross pollination (98.00 per cent), followed by self-pollination (94.00 per cent) and natural open pollination (84.00 per cent).

**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 (Farooqi & Sreeramu, 2004) and these fundamental information including anthesis, stigma receptivity, pollen viability and fertility, etc., are much needed for programming crop improvement through hybridization.

The flowers were scarlet red coloured with six, long radiating anthers and the style bent almost 90° at the point of attachment to the ovary. The stamen displayed profuse orange - yellow pollen. The pistil possessed three loculedovary which formed an ellipsoidal capsule. The individual flower weighed 2.52 g. Earlier floral description of G. superba was made by Farooqi & Sreeramu (2004), Rajamani et al. (2009) and Gupta & Raina (2001) and the reports are similar to the observations of this study.

In the present study, the number of days taken for completion of flowering phases in G. superba 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.

In G. superba, the perianth lobes at the bud opening stage were light greenish in colour. This was followed by the stigma receptive stage which was characterized by perianth lobes that were scarlet red at the tip, yellow in the middle and greenish towards the base. Post pollination stage was characterized by the upper half of perianth lobes being scarlet red and the lower portion being yellow coloured. Lastly, the perianth lobes turned entirely into scarlet red. This observation was on line with the findings of Farooqi & Sreeramu (2004) and Rajamani et al. (2009) (Plate 1)

In general, the duration of flowering phase was 21.10 days in G. superba. 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 G. superba 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 G. superba was between 8.30 to 10.30 am.

In the present study, the anther dehiscence 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) and Nagajothy (2008) 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. In G. superba, 97.50 per cent pod set 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 receptive 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. Similar reports were made by Rajagopalan (1994) and Gupta & 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.

Pollen morphology was studied under compound microscope by collecting samples of fresh pollen from G. superba and shape of the pollen was found as oval. 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.

The mean percentage of fertile pollen in G. superba was maximum on the day of anther dehiscence and declined gradually as the age of pollen increased. 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 per cent of pollen was viable even six hours after dehiscence (Plate 2). 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 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. superba, maximum pod set was observed in artificial cross pollination within the species followed by self-pollination. Minimum pod set was noticed in natural self or cross 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° 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 Rudre Gowda (1997). Low seed set under natural pollination was also observed by Gupta and Raina (2001) in G. superba.

Reference


Rajamani, K., R. Chitra, P. Pad Mapriya, K. Kumananan and Vadivel, E. 2009. Gloriosa taxonomy,


<table>
<thead>
<tr>
<th>Character</th>
<th>Dimension</th>
<th>SD</th>
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</thead>
<tbody>
<tr>
<td>Pedicel length</td>
<td>10.45(cm)</td>
<td>1.027</td>
</tr>
<tr>
<td>Tepal size</td>
<td>6.80 X1.31(cm)</td>
<td>0.660</td>
</tr>
<tr>
<td>Length of stamen</td>
<td>5.42(cm)</td>
<td>0.389</td>
</tr>
<tr>
<td>Length of the pistil</td>
<td>6.40(cm)</td>
<td>0.704</td>
</tr>
<tr>
<td>Flower weight</td>
<td>2.52(g)</td>
<td>0.464</td>
</tr>
<tr>
<td>Avg. pollen size</td>
<td>52.82(μm)</td>
<td>5.747</td>
</tr>
<tr>
<td>Pollen output (Nos.)</td>
<td>7,30,000</td>
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</tr>
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</table>

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

<table>
<thead>
<tr>
<th>Flower no.</th>
<th>Date of different flowering phase</th>
<th>Duration of flowering phase</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>11.9.11</td>
<td>25.9.11</td>
</tr>
<tr>
<td>2</td>
<td>15.9.11</td>
<td>30.9.11</td>
</tr>
<tr>
<td>3</td>
<td>20.9.11</td>
<td>1.10.11</td>
</tr>
<tr>
<td>4</td>
<td>22.9.11</td>
<td>7.10.11</td>
</tr>
<tr>
<td>5</td>
<td>26.9.11</td>
<td>7.10.11</td>
</tr>
<tr>
<td>6</td>
<td>30.9.11</td>
<td>15.10.11</td>
</tr>
<tr>
<td>7</td>
<td>2.10.11</td>
<td>14.10.11</td>
</tr>
<tr>
<td>8</td>
<td>7.10.11</td>
<td>20.10.11</td>
</tr>
<tr>
<td>9</td>
<td>10.10.11</td>
<td>23.10.11</td>
</tr>
<tr>
<td>10</td>
<td>15.10.11</td>
<td>26.10.11</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Table 3. Pod set under various types of pollination in *Gloriosa superba*

<table>
<thead>
<tr>
<th>Type of pollination</th>
<th>G. superba</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. of flowers taken for study</td>
<td>No. of pod set</td>
<td>% of pod set</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural open pollination</td>
<td>50</td>
<td>42</td>
<td>84.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self pollination</td>
<td>50</td>
<td>47</td>
<td>94.00</td>
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</tr>
<tr>
<td>Artificial cross pollination within the species</td>
<td>50</td>
<td>49</td>
<td>98.00</td>
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</tr>
</tbody>
</table>
**Fig. 1 Time of bud opening and anther dehiscence**

**Fig. 2 Stigma receptivity of *Gloriosa superba***

**Fig. 3 Pollen viability and fertility in *G. superba***
Plate 1. Flower developmental stages of *G. superba*

2a. Pollen morphology (20X magnification)  
2b. Pollen fertility (20X magnification)  
2c. Pollen viability (20X magnification)

Plate 2. Pollen morphology, fertility and viability