Research Article

# Floral Biology and Breeding in *Gloriosa rothschildiana*

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**Abstract**

Hybridization enables the gene transfer, which may lead to the additional source of variation for the desirable horticultural traits. An experimental study was carried out to study the flower morphology and tp understand the crossability relationship of *Gloriosa rothschildiana* with *Gloriosa superba*. *G. rothschildiana* crossed with five genotypes of *G. superba* in both directions. The flowers of *G. 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 pod growth of 2.00 cm length was observed within 25 days of pollination and there after shrinked irrespective of the cross combination. None of the pod reached the desired harvestable stage. Post fertilization barrier was observed in both direct and reciprocal crosses of gloriosa species. This may be due to embryos abortion and degeneration during embryogenesis which needs to studied.

**Keywords:** Glory lily, interspecific hybridization, post fertilization barrier, embryo rescue

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## Introduction

Glory lily (Colchicaceae) is a perennial tuberous climbing herb extensively scattered in the tropical and sub-tropical parts of the India, including the foothills of Himalayas. The genus *Gloriosa* comprises of 10 to 15 known species *viz*., *G. superba, G. lutea, G. plantii, G. latifolia, G. magnifica, G. rothschildiana, G. abyssinica, G. longifolia* and *G. simplex.* These species are distributed mainly in Africa. The important species found in India are *G. superba* and *G. rothschildiana* [1]. Various compounds have been isolated from the plant parts of glory lily mainly tubers and seeds, *viz* colchicine, colchicoside (its semi-synthetic derivative — thiocolchicoside), superbine, gloriosine, lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, N-formyl deacetylcolchicine [2]. Among them, colchicine and colchicoside are the major constituents, which are used to treat gout and rheumatism [3].

Another existing species *Gloriosa rothschildiana*, bearing 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 of Gloriosa *superba* have been recorded by the early workers like [4-10].

The growing demand for the seeds of *Gloriosa superba* in the international market and the wider popularity it has gained among the farmers necessitates attempts to induce new variability with qualitative and quantitative characters. The genetic variability in *Gloriosa superba* is low owing to the continued vegetative propagation through tubers which has reduced the vigour, tolerance to biotic and abiotic stress causing low yields. Traditional or conventional breeding has not been attempted so far as there is only one ecotype under cultivation and genetic wealth is limited. Introduction of new variability is the only option for the breeders at present to create new variability for selection of high yielding cultivars [11].

Hence there is a need to explore the possibilities for developing variability in this species with varying flower colour, shelf life, high seed yield and improved colchicine content through hybridization. Keeping the above facts in view a detailed study of flower morphology, pollen characteristics and hybridization in *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 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). The observations were also recorded on anther dehiscence, sigma receptivity, pollen viability and fertility and pollination behaviour.

**Table 1** Flower morphology and pollination behaviour of *Gloriosa rothschildiana*

| Flower morphological 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 |

**Table 2** Pollen viability study in *Gloriosa rothschildiana*

| Age of pollen grain (day) | | Pollen viability | 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

***Interspecific hybridization***

In a hybridization programme, to combine important traits of germplasm, five high yielding genotypes of *Gloriosa superba* [GS01, GS02, GS03, GS04, GS05] were crossed with *Gloriosa rothschildiana* [GR]. Ten cross combinations were made from the selected parents.

***Crossing technique***

A separate hybridization block was maintained for the production of $F_1$ seeds at Tamil Nadu Agricultural University, Coimbatore. During hybridization programme, flowers of female parent at pre-anthesis stage were emasculation between 7.00 and 9.00 am and bagged with butter paper cover. Similarly, in the male parents, selected flower buds at pre-anthesis stage were bagged without emasculation to avoid contamination by foreign pollen for collection of pollen grains. Pollen from bagged pollen parents were collected between 8.00 and 9.00 am in the next day morning and dusted on the stigma of emasculated flowers of the respective female parents. The flowers were bagged again with butter paper covers and labeled. The covers were removed after ensuring proper pod set. Observations were recorded on pod set percentage, days for pod maturity, fresh pod weight, dry pod weight, fresh seed weight per pod, number of seeds per pod (**Figure 1**).

## Results and Discussion

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 at pre-anthesis stage

Emasculation

Dusting of pollen

Bagging

**Figure 1** Crossing technique in *G. rothschildiana*

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). The flowering phase ranged from 15 to 24 days (mean of 20.70 days). The maximum mean percentage of 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). These descriptions are in accordance with the reports of [7], [12] and [9]. [12] also made similar reports about the different stages of flowering in *G. rothschildiana*. [5] and [9] also opined that the period of flower bud development extended upto 17 to 20 days depending upon the season.

***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 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. 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. [4], [5], [6], [8] and [9] also reported similar observation on anthesis in *G. superba*. Varying reports were made by [6] and [7] 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 characteristics***

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 in both the species. 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. The average pollen production per flower was 7,01,250 with average size of 65.68 µm. Based on the appearance of exine of the pollen grains, oval or elliptical shaped pollen were observed and similar findings were made by [5] and [13] who reported the presence of oval shaped pollen with smooth exine. In ref [14] also reported the presence of ellipsoidal pollen in the interspecific tetraploid hybrids of *Gloriosa*.

***Pollination behaviour***

*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. These findings are in accordance with the experiments of [4] [6] and [15]. Low seed set under natural pollination was also observed by [7] in *G. superba.*

***Interspecific incompatibility***

Self and cross incompatibility of a crop is a major constraint in hybridization programme. High cross compatibility of the chosen parents along with other desirable horticultural traits accelerates success of any controlled hybridization programme. Incompatibility may exist among genotypes, it is necessary to find out the compatible relationship of the selected genotypes before attempting any inter-varietal hybridization.

In the present study on interspecific hybridization, varying percentage of pod set was observed in the interspecific crosses between *G. rothschildiana* and the germplasm of *G. superba*. In all cross combinations involving these two species, ovary enlarges and the pod growth was observed upto a maximum of 2.00 cm length over a period of 25 days of pollination (**Table 3**). But thereafter, the pods started shrinking and none of the pods reached the harvestable stage (**Figures 2** and **3**). The data on pod set percentage among the interspecific crosses indicated that GR x GS02 recorded the highest pod set percentage (95.33), followed by GR x GS05 which registered 94.66 per cent. The cross GS05 x GR recorded the minimum pod set percentage (73.00 per cent). The mean pod length at 25 days after pollination was maximum (2.40 cm) in GS01 x GR, followed by GR x GS02 (2.20 cm) and GS04 x GR and GS05 x GR recorded the minimum pod length (1.50 cm). After 35 days of pollination, there was shrinkage of pods and the pod length recorded 1.80 cm in GS01 x GR. After 35 days, all pods dried prematurely and none of the pods reached the harvest stage.

The results showed that interspecific hybridization had a more serious post-fertilization barrier. The probable reason for the abortion might be due to delayed embryo abortion as the ploidy levels of these *G. rothschildiana* and *G. superba* varied as reported by [16]. The genetic architecture of *G. rothschildiana* was 2n=66 and that of *G. superba* was 2n=22 and delayed incompatibility existed between *G. superba* and *G. rothschildiana* 'Maron gold'.

The failure in the interspecific crosses may be due to the failure of fertilized ovules to develop into mature seeds [17]. The causative factors include the presence of lethal genes and failure or early breakdown of the developing endosperm. Failure or arrest of normal development of embryo and endosperm may also result from unfavorable interaction between the embryo and surrounding ovular tissues. Often, embryo breakdown is associated with proliferation of the nucellar or integumentary cells [18]. Failure of developing endosperm leading to death of potentially viable hybrid embryos is the common result of causes between closely related species [19].

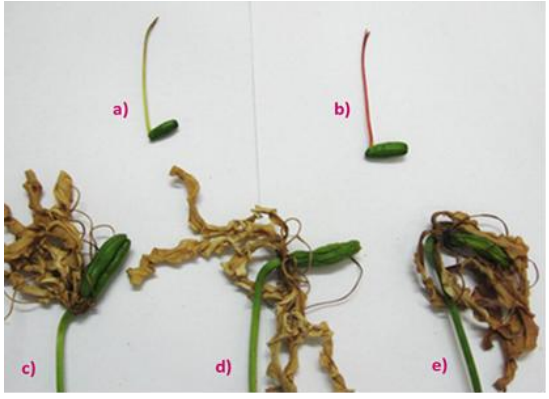


**Figure 2** Post fertilization barrier in interspecific hybridisation of *Gloriosa spp.*

**Table 3** Interspecific hybridization in *Gloriosa rothschildiana*

| S. No | Crosses | No. of flowers pollinated | Pod set percentage (%) | Pod length after 25 days of pollination (g) | Pod length after 35 days of pollination (g) | No of pods harvested |
|---|---|---|---|---|---|---|
| 1. | GS01 x GR | 149 | 82.55 | 2.40 | 1.80 | 0.00 |
| 2. | GR x GS01 | 110 | 88.18 | 1.80 | 1.30 | 0.00 |
| 3. | GS02 x GR | 150 | 91.33 | 1.90 | 1.40 | 0.00 |
| 4. | GR x GS02 | 150 | 95.33 | 2.20 | 1.73 | 0.00 |
| 5. | GS03 x GR | 100 | 75.00 | 2.10 | 1.70 | 0.00 |
| 6. | GR x GS03 | 60 | 81.66 | 1.80 | 1.30 | 0.00 |
| 7. | GS04x GR | 110 | 92.72 | 1.50 | 1.20 | 0.00 |
| 8. | GR x GS04 | 100 | 75.00 | 1.80 | 1.50 | 0.00 |
| 9. | GS05 x GR | 100 | 73.00 | 1.50 | 1.10 | 0.00 |
| 10. | GR x GS05 | 150 | 94.66 | 2.00 | 1.60 | 0.00 |

Note: Stating that pod developed aborted after 25 days of pollination

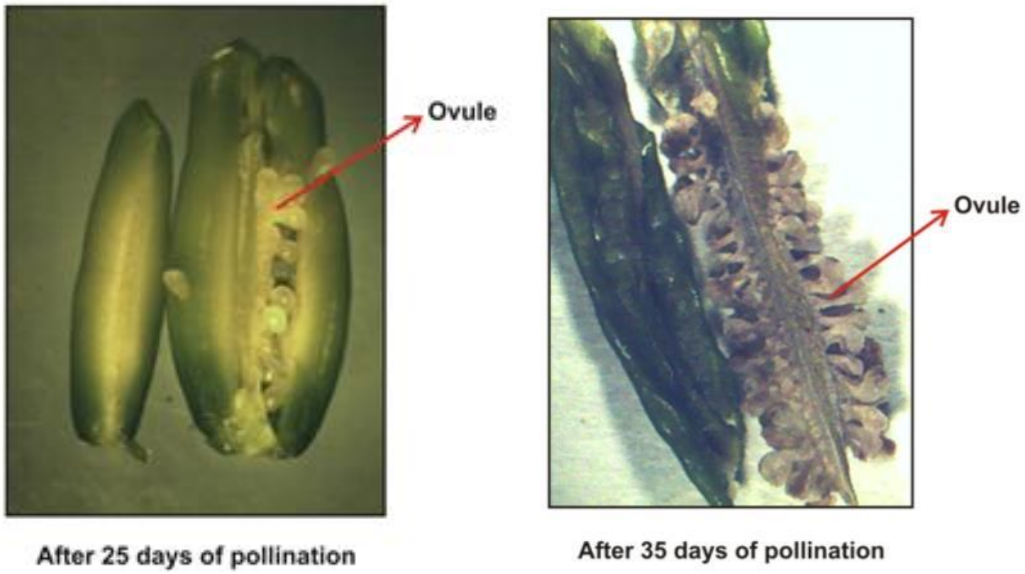


**Figure 3** Microscopic view of ovary in interspecific hybridisation

Interspecific crosses are usually hindered by various cross-incompatibility mechanisms, successful production of interspecific hybrids could be achieved only from limited crosses among those using many cultivars/strains of both parents, suggesting the importance of the selection of the compatible genotypes. Unilateral cross incompatibility is commonly observed in interspecific cross combinations, so reciprocal crosses should be conducted as an indispensable step [20].

The failure to pod development may also be due to post-fertilization barriers, in which hybrid embryos obtained after fertilization degenerate during their development, can sometimes be overcome by embryo rescue techniques such as ovule or embryo culture. In some ornamental plants, such as *Lilium* spp. [21], Primula spp. [22], *Cyclamen* spp. [23], interspecific hybrid cultivars have so far been produced *via* embryo rescue. Alstroemeria ovaries were collected 10-23 days after hand pollination and their ovules were bred with the assistance of in vitro techniques such as in ovulo embryo rescue [24]. In Colchicaceous ornamentals, intergeneric hybridization has recently been performed for widening the variability in horticultural traits: Santonia 'Golden Lights', an intergeneric hybrid cultivar of *Sandersonia aurantiaca* and *Littonia modesta*, was developed *via* ovule culture [25], [26], [27], [28]; intergeneric hybrids between *S. aurantiaca* and *Gloriosa* spp. were produced *via* ovule culture [29], [30], [28].

Hybrid inviability and weakness can be overcome by ovule/ovary culture *in vitro* to obtain successful interspecific hybrids in Colchicaceae. Reports of this suggestion are evident from the efforts made early researchers. The crossed ovary of *Sandersonia aurantiaca* and *Gloriosa rothschildiana* was cultured in half-strength (1/2) MS medium containing 0.01 mg $l^{-1}$*α*-naphthalene acetic acid (NAA) and 0.01 mg $l^{-1}$ 6-benzyladenine (BA) by [30].

Further, the follow up studies in this aspect for developing interspecific hybrid through *in vitro* ovary / ovule culture technique will be highly useful. Such a line of investigation and consequent development of interspecific hybrids will be useful, in widening the genetic base.

## Conclusion

The study helped in finding out the existence of interspecific incompatibility among Gloriosa species. The results may help the breeders to design breeding programme of Gloriosa species along with embryo rescue technique for utilization of genetic resources. To achieve precise information, large number of genotypes or species should be included in a hybridization programme.

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