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Floral biology of *Rauwolfia micrantha* Hook. f.: a rare and endemic medicinal plant of Western Ghats, India

R. Rama Subbu*, A.K. Sreekala and S.K. Kulloli

Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram 695 562, Kerala, India

Abstract

Rauwolfia micrantha Hook. f. is a rare and endemic medicinal plant of southern Western Ghats, India. It flowers throughout the year under the climatic conditions of Aryankavu, South India with the peak during May. Flowers open in the morning between 0500 and 0900h followed by anther dehiscence at 0600-1000h. The maximum receptivity of stigma was found on the day of anthesis. Pollen grains were round, light yellow in colour and with a mean diameter of 36.68µm. Fruits are single or double seeded drupes.

Keywords : anthesis, floral biology, pollen grains, *Rauwolfia micrantha,* stigma receptivity

INTRODUCTION

Rauwolfia micrantha Hook. f. (Apocynaceae) is a rare, endemic, woody, medicinal shrub distributed at an elevation up to 600m in the Tirunelveli and Travancore hills of the Western Ghats in southern India. The medicinal property of the plant is mainly due to the wide spectrum of alkaloids such as reserpine, reserpiline and serpentine in its roots (Anonymous, 1969). *R. micrantha* is used as a substitute for *R. serpentina* to treat a variety of nervous disorders in Indian traditional medicine (*Ayurveda*) especially in the state of Kerala, South India (Sahu, 1979).

Factors such as endemicity, restricted distribution, small populations in accessible areas, severe anthropogenic pressure on forest land and poor seed viability have caused the decline of *R. micrantha* in the world. It is reported to be rare (Sahu, 1979). The knowledge of floral biology is a prerequisite in estimating the overall reproductive potentiality of a species, which in turn controls adaptive changes in organisms (Simmonds, 1962). The studies on floral biology of plants have received much interest in recent years; but little attention has been given to rare and endangered plants species (Aspinwall and Christian, 1992). Due to its medicinal importance and narrow distribution, an attempt has been made to study the floral biology of *Rauwolfia micrantha*, which will be helpful for its successful plant hybridization programme.

MATERIALS AND METHODS

The study was carried out on *Rauwolfia micrantha* from Arayankavu (Kulathupuzha Forest Range, Kerala, India) between March 2006 and November 2007. Twenty healthy plants were selected in the community and observations were made on day to day basis on flowering season, flower development and anthesis. Peak flowering time was noted when maximum number of flowers opened. Fifty flower buds were selected at random and observations were made between 0400 to 1300h to study the time of anthesis and anther dehiscence. Pollen-ovule ratio was worked out as per the method suggested by Cruden (1977). Pollen fertility was assessed by using 2% acetocarmine and glycerin (1:1) staining technique. Pollen viability was checked by fluorochromatic reaction (FCR) test suggested by Shivanna and Rangaswamy (1992). To study the pollen germination *in vitro*, pollen grains collected from fresh flowers were incubated for 10 hours in a drop of Brewbakers medium containing 2% sucrose (Brewbaker and Kwack, 1963). Pollen grains which had produced pollen tubes longer than the diameter of the pollen grains were counted as viable. Stigma receptivity was studied visually with the help of hand lens and by hand pollination method. For the study of pollen germination on stigma (*in vivo*), the flowers were labeled at the time of anthesis. The pistils were collected from the labeled flowers at different time intervals after anthesis. *In vivo* pollen germination was checked by aniline blue fluorescent microscopic method as described by Shivanna and Rangaswamy (1992).

RESULTS AND DISCUSSION

R. micrantha, a medicinal shrub, starts flowering in the month of March and almost continuing flowering throughout the year with a peak during May (Fig. 1a). The flower buds take 10-15 days from initiation to full bloom. The inflorescence is a corymbose and consists of 20 ± 3.0 flowers. Flowers are small, bisexual, actinomorphic and hypogynous. Stamens 5, alternating with corolla lobes. Style slender, stigma capitate, wet and papillate. The ovary is bicarpellary syncarpous with one ovule in each carpel with axile placentation. The

*Corresponding Author
email: *balsams_india@yahoo.co.in*

flowers open in the morning between 0500 and 0900h. About 92 % of anthesis occurred between 0600 and 0800h and a maximum of 36 % was recorded during the first hour of anthesis (Table 1). Anther dehiscence was observed after one hour of opening of flowers and a maximum of 44 % was noticed between 0800 and 0900h. The anthers dehisced longitudinally and exposed pollen grains. The floral analysis indicated that each flower

Table 1. Anthesis and anther dehiscence in *Rauwolfia micrantha*

Time (hrs)	No. of flowers opened (out of 50)	% of flowers opened	No. of flowers with anther dehisced	% of flowers with anther dehisced
0400-0500	0	0	0	0
0500-0600	12	24	0	0
0600-0700	18	36	8	16
0700-0800	16	32	14	28
0800-0900	4	8	22	44
0900-1000	0	0	6	12
1000-1100	0	0	0	0
1100-1200	0	0	0	0
1200-1300	0	0	0	0

has five anthers and two ovules. A single anther contains on an average 1200 pollen grains and thus a flower has around 6000 pollens. Hence, P-O ratio is worked out to be 3000 pollens per ovule (3000:1), which confirms the pollination by entomophilly (Cruden, 1977). Pollen grains are round with a mean diameter of 36.68 µm. The acetocarmine staining technique reveals that 86% of pollen grains are fertile. Pollen viability by FCR test confirmed that 82 % of pollen grains were viable on the day of anthesis, which gradually decreased on successive days after anthesis. The quality of pollen was assessed on the basis of viability and vigour and the probability it may deliver functional sperm cells to the embryo sac following compatible pollination (Shivanna *et al*., 1991). The pollen vigour plays a significant role in pollen competition and pollen selection during pollen-pistil interaction (Ottaviano and Mulcahy, 1989). *In vitro* pollen germination studies indicated that the best pollen germination (92%) along with 1269 µm tube elongation was recorded in Brewbakers medium containing 2 % sucrose after 10 hours of incubation. Successful seed sets and establishing newer population generally depends upon viable pollen grains. In the present investigation, Brewbakers medium was found to be the most suitable medium for pollen germination in *R. micrantha.* Sucrose is reported to act as a nutritive material for pollen germination and it helps in maintaining proper osmotic balance between germination media and pollen cytoplasm (Johri and Vasil, 1961). Besides the medium contains carbohydrates, boron and calcium, which are other important substances that are required for pollen germination and subsequent tube elongation (Brewbaker and Kwack, 1963).

The stigmas remained receptive at the time of flower opening and hence it is protogynous in nature. To confirm the receptivity of stigma, hand pollinated flowers were observed periodically for seed setting. The visual observations indicated that the stigma was wet and shiny on the day of anthesis, retained its status for 48h, after which it turned black. This observation was further supported by *in vivo* pollen germination tests (Table 2 and Fig.1d). The results indicated that the maximum stigma receptivity of 70 % was recorded on the day of anthesis and it had lasted for 48 hours giving different percentage of pollen germination on stigma. Highest percentage of pollen germination (62 %) was recorded after 12h of anthesis which resulted in 58 % fruit set (Table 3 and Fig. 1b). On the contrary, the percentage of pollen germination declined considerably when pollinated after 24h of anthesis which resulted in 20 % fruit setting and thereafter no fruit set was observed. Stigma receptivity and *in vivo* pollen germination gradually decreased on successive days after anthesis.

Table 2. *In vivo* pollen germination before and after anthesis in *R. micrantha.*

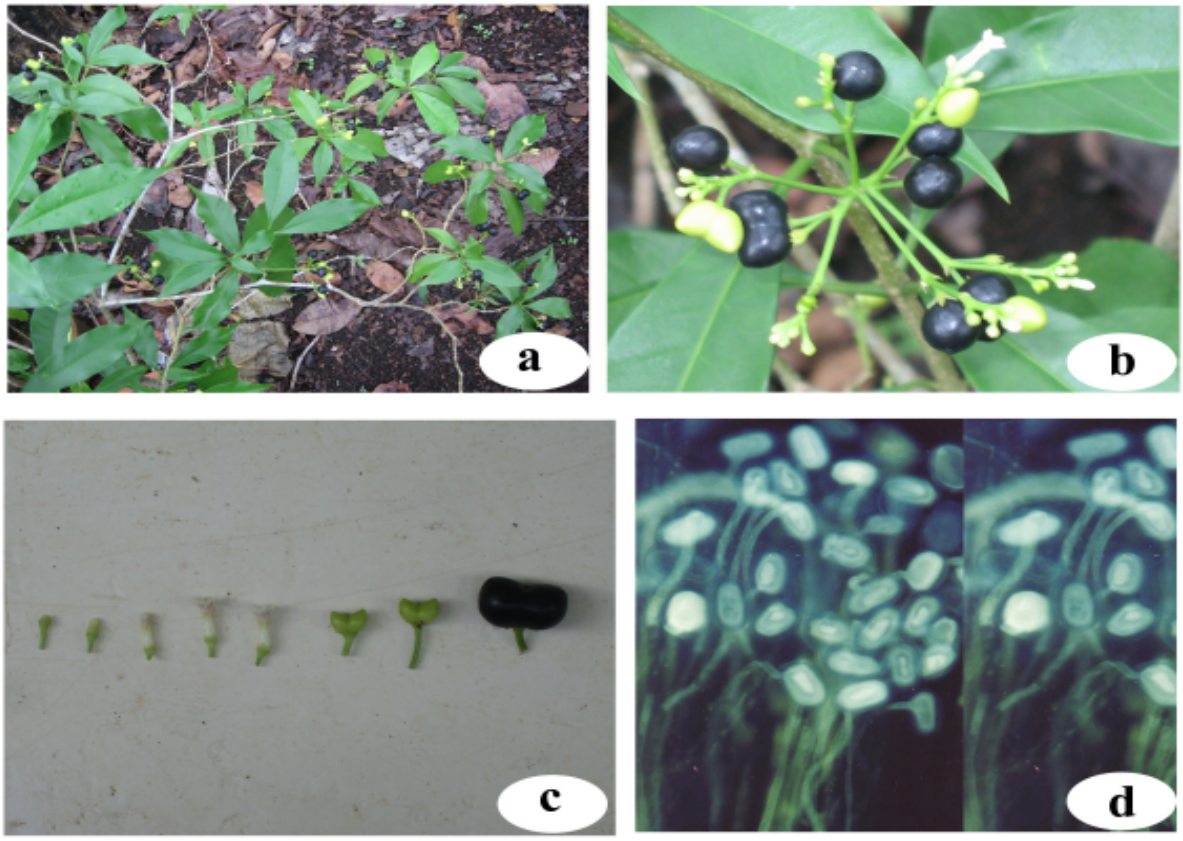
Time (hrs.)	% of pollen germination	Pollen tube length (µm)
6hrs before anthesis	0	0
At the time of anthesis	26	120
6hrs after anthesis	38	146
12 hrs after anthesis	62	340
18hrs after anthesis	44	220
24 hrs after anthesis	42	280
30 hrs after anthesis	36	160
36 hrs after anthesis	24	116
42 hrs after anthesis	20	110
48 hrs after anthesis	14	96

Table 3. Stigma receptivity and fruit set in *R. micrantha*

Period	No. of flowers pollinated	No. of flowers set fruits	% of fruit set
One day prior to anthesis	50	0	0
On the day of anthesis	50	24	58
One day after anthesis	50	10	20
Two days after anthesis	50	0	0

Findings of this study are similar to the observations of Sreekala *et al*. (2003) on *Ixora agasthyamalayana*, where maximum stigma receptivity was observed on the day of anthesis with 30 % fruit set, followed by 10% on the next day and thereafter no fruit setting. About 5 % of the flower buds and 10% of open flowers were infested by caterpillars and insect larvae. The insect larvae damaged the floral parts, which in turn adversely affected the fruit production.

Figure.1



(a) - *Rauwolfia micrantha*, in flowering
(b) - Fruiting branch with single and double seeded drupes
(c) - Flower development
(d) - Pollen germination on the stigmatic surface.

Pollen germination and subsequent post pollination events depends upon the receptivity of the stigma, its nature and compatibility. Stigma receptivity is a critical factor for successful completion of post-pollination events. Usually it is maximum soon after anthesis but it varies from species to species depending upon temperature and humidity (Shivanna and Johri, 1985). But in the candidate species, the stigmas remained receptive at the time of the flower opening and the pollen grains are well adhered on the stigmatic surface. The adhesion of pollens on the stigma is a primary requirement for successful pollination. When a pollen is accepted, a pollen tube comes out and grows towards the stigmatic pellicle by penetrating the cuticle and move downward (Shivanna, 1977). But if the pollen is incompatible, it does not germinate and seems to be rejected (Dickinson and Lewis, 1973). In *R. micrantha,* pollen tubes penetrate the stigmatic surface and reached up to the ovary and successfully fertilized the ovules. The fertilized ovules developed into seeds. The fruit development took 25-30 days for attaining maturity after fertilization. The fruits are single or double seeded drupes (Fig. 1b). The percentage of seed germination was only 20 %, which may be one of the reasons for its restricted distribution.

CONCLUSION

For biodiversity conservation and restoration, research on reproductive biology can play an important role in understanding the physical barrier that leads to population reduction of a species in the wild. Dependence on a specialized habitat, over exploitation due to its medicinal importance, low percentage of seed germination and floral damage caused by the insects could be the reasons for the limited distribution of *Rauvolfia micrantha* in the wild. Results of the present study on its floral biology would help in developing strategies to preserve the genetic potential of this rare plant and might prove to be crucial for its restoration and reintroduction.

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