



Floral and reproductive biology of *Sarpagandha Rauvolfia serpentina* (Gentianales: Apocynaceae) in semi-arid environment of India

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Abstract: Sarpagandha plant *Rauvolfia serpentina* (Linn.) Benth., ex Kurz bears small, tubular white to pinkish flowers with gamopetalous corolla, containing nectar deep at the base of the corolla tube. Psychophilous mode of pollination appears to be prevalent. Flowering occurs during two summer months. Anthesis takes place in the morning when atmospheric temperature ranges from 25-29 °C, and anther dehiscence from 28-31 °C. Flower longevity is for a little more than two days. Nectar is produced on both the days of flower opening, and over a wide range of ambient temperature (29-44 °C). Flowers are protogynous preventing selfing. Pollen viability and stigmatic receptivity are for a short duration. When compared with the 'absolute reproductive potential', the 'realized reproductive potential' is very low.

Keywords: Fertility status, floral biology, pollination, psychophily, reproductive potential, *Rauvolfia*, sarpagandha.

Sarpagandha (*Rauvolfia serpentina* (Linn.) Benth., ex Kurz.) is a medicinal plant par excellence (Blackwell 1990), producing useful alkaloids like reserpine (Sahu 1983). Various parts of this plant are used to

treat human ailments (Dutta & Virmani 1964; Farooq 2005; Ebadi 2007), in alternative systems of medicine. Sarpagandha is a threatened species found in the sub-tropical regions. Seed propagation is considered to be the best method for raising commercial crop, though seed production is highly variable and low (Bhadwar et al. 1956). To understand the probable reasons for low seed yield and threatened status of this plant, the present study was undertaken.

Materials and methods

Sarpagandha seeds were sown in the field in mid November 2005 and a nursery was raised at the Research Farm of CCS Haryana Agricultural University, Hisar (India). Ten-week old plants from the nursery were transplanted in small plots in the last week of January (on 24 January, 2006) and the plants were raised according to the prevailing agronomic practices (Image 1).

(i) Floral morphology and pollination mechanism: The functional relationship between floral morphology and probable pollinators was studied by recording floral attributes like number and placement of floral parts; and structure and position of the ovary. Corolla length and breadth/diameter were also measured (n = 50 in each case).

(ii) Floral phenology: Dates when the first flowering appeared marked commencement whereas complete absence of flowers on the plants marked cessation of flowering. Based on this, the flowering period was determined (n = 100 plants). Dates of mediocre and peak flowering (number of flowers per m²) were recorded by visual observations. Temperature maxima and minima during these periods were also recorded.

The time of anthesis, anther dehiscence and nectar production were recorded by confirming the

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Image 1. Sarpagandha *Rauvolfia serpentina* in bloom



Image 2. An inflorescence of Sarpagandha plant

opening of flower, presence of pollen and nectar in the newly opened flowers till their shedding ($n = 100$ respectively). Observations were repeated at weekly intervals till cessation of flowering. Longevity of the flower was determined by recording the time of opening and shedding ($n = 900$; flowers taken at each weekly interval = 100, total weekly observations during flowering period = 9).

(iii) Fertility status: Pollen was applied by hand on the stigma in the morning after anther dehiscence with the help of a fine brush. The lay out plan for hand pollination experiments was as under:

(a) Self-pollination experiments (Pollination within the same flower)

- Pollen (Day 1 flower) x Stigma (Day 1 flower)
- Pollen (Day 2 flower) x Stigma (Day 2 flower)

(b) Cross-pollination experiments (Pollination between flowers of same plant)

- Pollen (Day 1 flower) x Stigma (Day 1 flower)
- Pollen (Day 1 flower) x Stigma (Day 2 flower)
- Pollen (Day 2 flower) x Stigma (Day 1 flower)
- Pollen (Day 2 flower) x Stigma (Day 2 flower)

Each experiment was repeated on 50 sets of flowers and observations were recorded on seed sets in the flowers receiving pollen.

(iv) Duration of pollen viability and stigmatic receptivity: Pollen from the controlled/guarded flowers was applied on the stigmas of first day and second day at an interval of 0, 2, 4, 6, 8, 10 hours after liberation (i.e. at 0600, 0800, 1000, 1200, 1400, 1600 hr on the first day and at 0600, 0800, 1000 and 1200 hr on the second day). Seed set was recorded in 30 sets

of flowers in each case. Recipient flowers exhibiting seed set confirmed viability of pollen/receptivity of stigma.

(v) Absolute and ecological reproductive potential: Number of inflorescences per plant, flowers per inflorescence ($n = 50$ in each case), ovaries per flower, locules per ovary, and ovules per locule ($n = 100$ in each case) were recorded. The absolute/maximum reproductive potential of the plant (its inherent capability to produce seeds) was derived by multiplying the average values of these attributes.

To determine the ecological/realized reproductive potential of Sarpagandha, 50 plants were selected randomly in the field and marked. On maturity, plants were harvested individually, seeds were taken out and counted in a seed counter. The ecological/realized reproductive potential (number of seeds produced per plant) of this plant was determined accordingly.

Results and Discussion

(i) Floral morphology and pollination mechanism: Inflorescence of Sarpagandha is terminal and consists of small flowers in compact cymes forming a hemispherical head at the end of a long peduncle (Image 2). Flowers are small, pedicillate, complete and hermaphroditic with five deep red and glabrous sepals. Five petals in gamopetalous condition form a tubular corolla which is swollen in the middle and white to pink in colour. Corolla tube measure 17.7 ± 0.22 mm (mean \pm SD, $n = 50$) in length and $2.52 \pm$

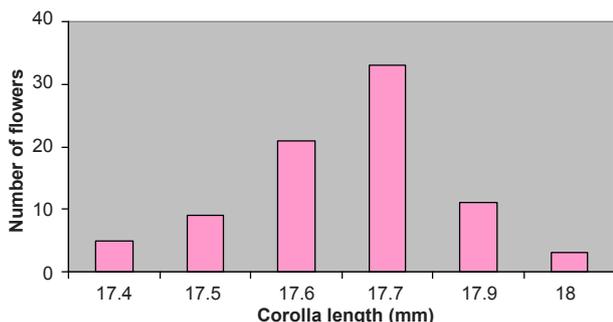


Figure 1. Frequency distribution of corolla length in Sarpagandha

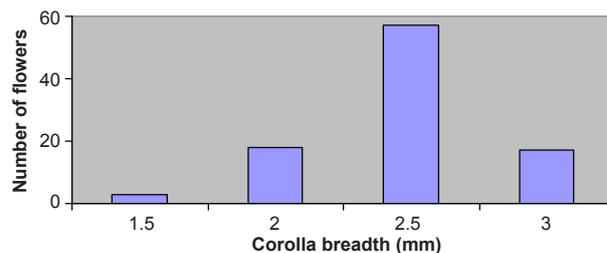


Figure 2. Frequency distribution of corolla breadth in Sarpagandha

0.79 (mean ± SD, n = 50) mm in breadth / diameter (Figs. 1 & 2). Five stamens in epipetalous condition are enclosed within the dilated portion of the corolla tube. Two connate carpels have a filiform style and large bifid stigma; a bilocular ovary has two ovules in each locule. The flowers of Sarpagandha have highly narrow and long tubular corolla. Such flowers make them a perfect representative of psychophilous pollination syndrome negating all other syndromes (Barrows 1976; Schemske 1976; Faegri & van der Pijl 1979; Suzuki et al. 1987; Sihag & Kaur 1997).

(ii) Floral phenology: Flowering occurred during peak summer, last week of May, when maximum & minimum temperatures ranged between 35.2-43 °C and 20-29 °C, and within eight days on all the Sarpagandha plants (Table 1). Flowering remained mediocre (quantified in terms of numbers / m²) for about two weeks from early June to mid June when maximum and minimum temperatures ranged between 33.4-42.7 °C and 20.5-28.5 °C, respectively. The peak flowering, continued for 43 days, when ambient temperature fluctuated between 31-40.9 °C maximum and 20.9-30.7 °C minimum. Thereafter, decline started till

cessation in the first week of August when maximum and minimum temperatures fluctuated between 32.6-36.3 °C and 25.0-26.5 °C. Thus, the plant remained in blooming stage from last week of May to first week of August over wide range of temperature (Table 1). Temperature dependent floral phenology has been reported earlier also (Ramani 1995; Sihag & Kaur 1995; Sihag & Priti 1997).

Flowers started opening in the early morning between 0500-0530 hr when ambient temperature fluctuated between 24-29 °C (Table 2). However, anthers did not dehisce on the first day of flowering; it took place on the second day of flower opening between 0700-0730 hr at relatively higher temperature range of 28-31 °C. Under the semi-arid sub-tropical conditions of Hisar, flower longevity was a little more than two days - ranged between 54-58 hr (mean ± SD = 56.53 ± 2.20, n = 900). Nectar secretion started on the first day of flower opening between 0800 to 0830 hr in the morning and continued up to 1300 to 1330 hr in the evening; it again started between 1500 to 1530 hr to continue till dusk at 1730hr on both the days. Nectar production was for a longer time and at wider range of temperature, 27 to 44 °C (Table 2). The diurnally opening tubular flower with liberation

Table 1. Duration of different parameters of Sarpagandha plant in relation to ambient temperature.

	Plant parameters	Time intervals (dates)	Duration (days)	Ambient temperature (°C)			
				Ranges		Averages	
				Max.	Min.	Max.	Min.
1	Transplantation	24.i.2006	--	22.7	9.0	22.7	9.0
2	Commencement of flowering ^a	24.v.2006 to 31.v.2006	8	35.2-43.0	20.0-29.0	40.3	25.2
3	Mediocre flowering ^a	01.vi.2006 to 17.vi.2006	17	33.4-42.7	20.5-28.5	38.3	24.5
4	Peak flowering ^a	18.vi.2006 to 31.vii.2007	43	31.0-40.9	20.9-30.7	36.7	25.6
5	Cessation flowering ^a	05.viii.2006 to 12.viii.2006	8	32.6-36.3	25.0-26.5	34.2	25.9

^a - Observations made on 100 plants

	Floral parameters	Time range (hr)	Duration (hr)	Ambient temperature ranges (°C)
1	Flower opening ^a	0530-0600	1/2	25-29
2	Anther dehiscence ^a	0700-0730	1/2	25-29
3	Nectar production ^a	0800-1300, 1500-1730	7.30	27-44
4	Flower longevity	54-58	56.5 ± 2.2 ^b	25-44

^a - Observations made on 100 flowers; ^b - Mean ± SD of 900 observations

Table 2. Different floral parameters of Sarpagandha in relation to ambient temperature.

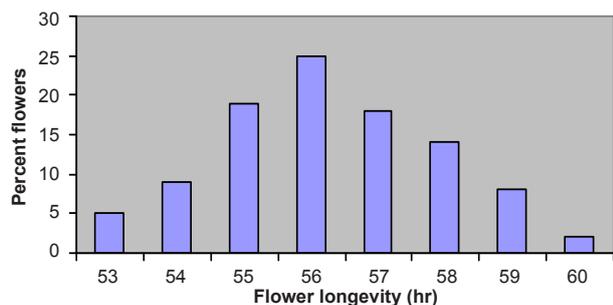


Figure 3. Frequency distribution of flower longevity in Sarpagandha

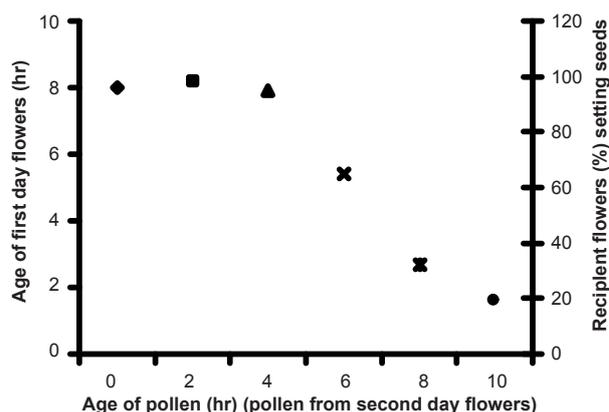


Figure 4. Level of pollen viability and/ or stigmatic receptivity (measured by per cent of flowers setting seeds) after different time duration in Sarpagandha.

of floral reward during day time further makes it an example of psychophilous pollination syndrome (Faegri & van der Pijl 1979; Sihag & Kaur 1997). The flower longevity was very short (56.5h, n = 900, Table 2, Fig. 3), most probably due to the very high ambient temperature regime.

(iii) Fertility status: As pollen was not available on the first day of flower opening, self-pollination was not possible on day one. Whether stigma was receptive or not on the day of flower anthesis, could not be ascertained. On the second day, after anthesis self-pollen was available, but self-pollination resulted in no seed set. Either stigma was not receptive in second day flowers or due to self-incompatibility in the flowers. These possibilities were tested through cross-pollination experiments. Crossing also was not possible between any two first day flowers as well as between a first day flower as pollen donor and a second day flower as recipient due to non-availability of pollen in the donor flower. Cross-pollination between second day flower as pollen donor and second day flower as pollen recipient also resulted in no seed set. This again indicates self-incompatibility or non-receptivity of stigma on the second day. However, cross-pollination between first day flower as pollen recipient (stigma) and second day flower as pollen donor (pollen available) produced seeds in 100% recipient flowers,

indicating protogyny in Sarpagandha. However, fresh self-pollen (from second day flower) could not fertilize the ovary of second day flower confirming that stigma was not receptive on the second day after anthesis. These experiments revealed that in the two days' age of flower, its stigma was receptive only on the first day as anthers dehisced on the second day when stigma had become non-receptive. Therefore, flowers of Sarpagandha need to be cross-pollinated as in umbelliferous plants (Sihag 1985a), onion (Sihag 1985b) and all cultivars of litchi (*Litchi chinensis* Sonn.) (Ray & Sharma 1995).

(iv) Duration of pollen viability and stigmatic receptivity: The pollen of Sarpagandha remained almost fully viable and stigma fully receptive only for four hours (Fig. 4). Thereafter, viability of pollen and/or receptivity of stigma declined. These were low after eight hours and very low after 10 hours. On the next day, flower completely lost stigmatic receptivity, even fresh pollen did not produce seeds in the pollen-recipient flowers. Therefore, pollination has to be completed on the first day itself, that too within a short period (< 6hr).

Table 3. Values of different floral attributes of sarpagandha determining its absolute and ecological reproductive potentials

	Floral attribute	Value ^a
1	Number of inflorescences on a plant (I) ¹	40.5 ± 22.8
2	Number of flowers in an inflorescence (F) ¹	45.4 ± 9.07
3	Number of ovaries per flower (O) ²	1 ± 0
4	Number of locules per ovary (L) ²	2 ± 0
5	Number of ovules per locule (S) ²	2 ± 0
6	Absolute/maximum reproductive potential (Rm) ³	7355
7	Ecological /realized reproductive potential (Re) ⁴	3178 ± 356

^a - Mean ± s.d.; ¹ - n = 50; ² - n = 100; ³ - Derived by multiplying values of 1-5 attributes; ⁴ - Derived from number of seeds produced per plant (n = 50)

(v) Absolute and ecological reproductive potential: The number of inflorescences per plant ranged from 17 to 63 (mean ± SD = 40.5 ± 22.8, n = 50) and flowers in the inflorescences ranged from 36 to 54 (mean ± SD = 45.4 ± 9.07, n = 50). Each flower produced four seeds (n = 100) on an average, and the normal Sarpagandha plant indicates a potential to produce 7355 seeds which is the absolute / maximum reproductive potential (Rm) (Table 3). The ecological/realized reproductive potential (Re) under Hisar conditions was, only 43.2% (3178 ± 356 seeds per plant) of its absolute/maximum reproductive potential (Table 3). This may be due to the presence of some strong ecological constraint(s). Factors responsible for low ecological reproductive potential remain to be investigated.

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