



FLORAL BIOLOGY AND POLLINATION OF *Solanum sisymbriifolium* Lamk.

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ABSTRACT

The present paper deals with flower morphology, anthesis, pollen production, pollen ovule ratio, foraging behaviour of flower visitors, pollen germination (*in vitro* and *in vivo*) and stigma receptivity of *Solanum sisymbriifolium* Lamk. belonging to the family Solanaceae, which is an important medicinal plant. The flowers open in between 5.00 to 7.00 hrs. As soon as flowers open, different insects like Thrips, *Ceratina viridissima*, *Apis* sp., *Amegilla* sp., *Xylocopa* sp. and few members of Lepidoptera, Diptera and Coleoptera visit flowers to collect forage materials and help in pollination. A single flower produces an average of 4,475,266 pollen grains and pollen ovule ratio was obtained 14918:1. The maximum (90%) pollen germination along with 1305µm tube development was observed in 20% sucrose solution supplemented with 100ppm boric acid. *In vivo* pollen germination showed that the stigma receptivity was maximum (70%) at first day after anthesis and showing 90.54% *in vivo* germinating pollen along with 1406µm pollen tube over stigma head.

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INTRODUCTION

The knowledge of floral biology, including pollination is prerequisite for any rational breeding programme and determination of extent of seed and fruit setting. Seeds and fruits are the economic products of more than 90% of flowering plants. Good fruit set and high crop yield generally depend on viable pollen grains. The objective of the present investigation is to find out the floral biology, anthesis, pollen production, foraging behaviour of flower visitors and *in vitro* pollen germination of *Solanum sisymbriifolium* belonging to the family Solanaceae, which is a medicinally important plant.

MATERIAL AND METHODS

Morphology of acetolysed (Erdtman, 1960) pollen and receptive stigmas (Ciampolini *et al.* 1996) were studied by Scanning Electron Microscope at USIC, Burdwan University. For the SEM of pollen and stigmas, these were collected during receptive period, washed in 0.015M phosphate buffer (pH 7.2), fixed with 2.5% glutaraldehyde for 2 hrs. dehydrated in ethanol series and finally critical point dried prior to gold coating. Microphotographs were taken from S-530 Hitachi Model. Flowering period, flower colour, odour and other floral characters were visually observed through extensive field exploration. Anthesis and other phenological characters were studied following the method of Reddi and Janaki Bai (1981) and Mathur and Mohan Ram (1986). Pollen productivity, pollen-ovule ratio estimation and role of flower visitors in pollination were studied following the procedure as suggested by Mandal and Chanda (1981) and Shivanna and Rangaswamy (1993). *In vitro* pollen germination was conducted using ten plants at random of the same species and same age located at different places on the University campus and areas of Santiniketan, Birbhum (87°41' and 87°42' east and 23°42' north latitude), were selected and observed for a period of 3 years. *In vitro* pollen germination was conducted to the effect of different nutrients like sucrose and boric acid at various concentrations.

The experimental set up was done as per the method of Shivanna and Rangaswamy (1993). Stigma receptivity and *in vivo* pollen germination were measured following the procedure of Joshi Rao and Saoji (1989) using 0.05% aniline blue dissolved in 0.05M NaH<sub>2</sub>PO<sub>4</sub> as staining reagent for *in vivo* pollen tubes. The flower visitors observed to pollinate the flowers were collected, preserved in our laboratory and identified using the specimens that were already identified from Zoological Survey of India, Kolkata. Insect visits were observed visually at 2 hrs. intervals in each day. Contributions of flower visitors to fruit setting were quantified by comparing fruit set between bagged and unbagged flowers. Bagging and netting of flowers was done at the bud stage. For these observations ten different plants from the same locality and ten different inflorescence of a single plant were taken.

RESULTS

Floral biology and Pollination Mechanism

Flowers (27.50 × 12.10 mm) are in axillary cymose on a long peduncle, pedicellate (5.0 - 7.5 mm), complete, actinomorphic, bisexual, hypogynous, white; sepals – 5, gamosepalous, spiny, persistent, green; petals – 5, gamopetalous, acute, united at the base, more or less ovate, white; stamens – 5, epipetalous, filament short (1.0 - 1.5 mm), stout, slightly swollen at the base, whitish, anther more or less oblong (8-9 × 1-2 mm), 2-celled, basifixed, dehiscence by apical pores, yellow; carpels – 2, syncarpous; ovary (1.7 – 2.0 mm), style long, slender (8.0 - 8.5 mm), stigma capitate, bifid (0.5 – 1.0 mm), apart from the anthers; ovary 2 chambered with many ovules in each chamber; fruit a berry with persistent calyx. A single flower produces an average of 4,475,266 pollen grains with the occurrence of 14918 pollen per ovule (Table 1). Pollen grains are 3-colporate, prolate, P/E ±36.00 × 26.40µm, polar out line triangular, equatorial outline elliptic, colpi linear, ±30.00µm long and ±1.20µm wide at the equator, both end tapering, exine ±1.85µm thick, sculpturing punctate-gillate (Fig. 1). Flowers are white, large in extra axillary cymose on a long peduncle. Flowers open early in the morning (5.00-7.00 hrs.) and have yellow coloured oblong anther which are dehisced by apical

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pores (Table 1). After flower opening *Apis* sp., *Ceratina viridissima*, *Amegilla* sp., *Xylocopa* sp., *Camponotus compressus* (ant), Thrips and members of Lepidoptera, Diptera and Coleoptera visit the flowers for collecting their forage materials. Bees like *Apis* sp., *Ceratina viridissima*, *Amegilla* sp. continuously visit the flowers in purpose of searching their food materials and during this time the pollen grains are adhered to the body parts of them and transferred to another receptive stigmas. *Xylocopa* sp. may be considered as effective pollinators to this plant. They use "buzz pollination" technique for collecting pollen from the poricidal anthers. The thrips are also capable to enter the flower even in bud stage. They feed on pollen, nectar and stigmatic exudates. They crawl through style to stigma and help in pollination. Diptera and Coleoptera also used to forage flowers which may or may not play the roll in pollination (Table 2) (Fig. 3,4,5,6). The fruit yielding potentiality of open flowers are higher (35%) than netted flowers (2.12%) or bagged flowers (1.50%) (Table 1).

**Table 1: Floral biology of *Solanum sisymbriifolium*.**

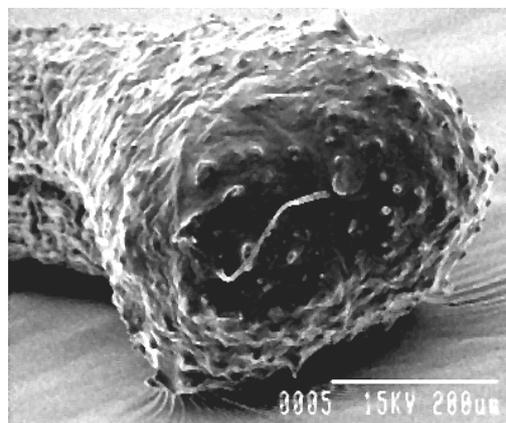
Floral parameters	Observations
Flowering period	Almost throughout the year
Flower shape	Regular
Flower size	27.50 × 12.10 mm.
Flower colour	White, with yellow anther
Odour	Present
Nectar	Present
Flower opening time	Early morning (5.00 – 7.00 hrs.)
Anther dehiscence time	Just after flower opening
Mean no. of anther per flower	5
Mode of anther dehiscence	By apical pores
Mean no. of pollen per anther	895053
Mean no. of pollen per flower	4475266
Mean no. of pollen per ovule	14918
Pollen morphology	3-colporate, prolate, ±36.00×26.40µm, colpi linear, ±30.00 x 1.20 µm, exine ±1.85µm thick, punctitigillate.
Stigma type	Wet type
Fruit-setting:	
Natural open flowers	35 %
Netting flowers	2.12 %
Bagging flowers	1.50%

**Table 2: Flower visitors of *Solanum sisymbriifolium***

Name of flower visitor	Visiting time	Forage matter
Thysanoptera		
Thrips	Day and night	Pollen and nectar
Hymenoptera		
Apidae		
<i>Apis</i> sp.	Day	Pollen and nectar
<i>Ceratina viridissima</i>	Day	Pollen and nectar
Anthophoridae		
<i>Amegilla</i> sp.	Day	Pollen and nectar
Xylocopidae		
<i>Xylocopa</i> sp.	Day	Pollen and nectar
Formicidae		
<i>Camponotus compressus</i>	Day and night	Pollen and nectar
(Ants)		
Lepidoptera		
Nymphalidae	Day	Nectar
Papilionidae	Day	Nectar
Pieridae	Day	Nectar
Diptera	Day	Nectar
Coleoptera	Day	Nectar



**Fig. 1: Pollen from equatorial view (SEM – 3000X)**



**Fig. 2: Stigma (SEM – 200X)**



**Fig. 3: *Ceratina* sp. visiting the flowers**



**Fig.4: *Amegilla* sp. visiting the flower**



**Fig.5: *Xylocopa* sp. visiting the flower**

**In vitro pollen germination**

20% sucrose solution showed the germination of pollen up to 50% (Table 3) and pollen tube development to 480 $\mu$ m and decreased the percentage of germination and tube length at higher concentrations. 100 ppm boric acid solution showed 60% (Table 4) germination and pollen tube development of 520 $\mu$ m. It is also observed that the addition of boric and sucrose solution results in the increase of pollen germination percentage as well as pollen tube development. The optimum result was obtained in 20% sucrose solution supplemented with 100 ppm boric acid showing 90% germination and 1305 $\mu$ m pollen tube development (Table 5). 300 ppm MgSO<sub>4</sub> solution showed 48% germination with 274 $\mu$ m tube development. (Table 6) (Fig. 7).

**Stigma receptivity and in vivo pollen germination**

Stigma is capitate, slightly bifid and showing heteromorphism. Thick walled, elongated cells are compactly arranged on the stigma head. Spiny texture is found over the stigma surface. Intercellular spaces are present moderately. Vascular strands reaches up to stigma head. Pollen grains are adhered to spiny surface of stigma head. Stigma is of wet type (Fig. 2). A critical observation on the stigma characters taking successive days after anthesis revealed that stigmas remained receptive more (70%) during first day after anthesis and showed 90.54% *in vivo* germinating pollen along with 1406 $\mu$ m pollen tube over stigma head (Table 7, Fig. 8, 9).

**Table 3: Effect of sucrose on in vitro pollen germination of *Solanum sisymbriifolium***

Concentration (%)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Pollen Tube Length ( $\mu$ m)	Germination (%)	Pollen Tube Length ( $\mu$ m)	Germination (%)	Pollen Tube Length ( $\mu$ m)
1	-	-	-	-	5	85
5	5	78	8	90	12	112
10	12	95	20	110	25	185
15	22	135	30	195	35	289
20	33	325	42	395	50	480
25	15	221	25	275	30	305
30	8	128	12	175	22	212

**Table 4: Effect of boric acid on in vitro pollen germination of *Solanum sisymbriifolium***

Concentration (ppm)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Pollen Tube Length ( $\mu$ m)	Germination (%)	Pollen Tube Length ( $\mu$ m)	Germination (%)	Pollen Tube Length ( $\mu$ m)
50	12	195	25	250	35	312
100	45	305	50	415	60	520
200	25	295	38	325	45	416
300	20	185	25	215	30	286
500	15	85	22	105	25	195

**Table 5: Effect of sucrose and boric acid on in vitro pollen germination of *Solanum sisymbriifolium***

Concentration (% + ppm)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Pollen Tube Length ( $\mu$ m)	Germination (%)	Pollen Tube Length ( $\mu$ m)	Germination (%)	Pollen Tube Length ( $\mu$ m)
20 + 50	35	535	40	782	50	985
20 + 100	65	975	75	1115	90	1305
20 + 200	37	525	39	675	45	825
20 + 300	18	325	25	530	32	612
20 + 500	15	135	20	221	25	310

**Table 6: Effect of different salts on in vitro pollen germination of *Solanum sisymbriifolium***

Concentration (ppm)	Ca(NO <sub>3</sub> ) <sub>2</sub>		KNO <sub>3</sub>		MgSO <sub>4</sub>	
	Germination (%)	Pollen Tube Length ( $\mu$ m)	Germination (%)	Pollen Tube Length ( $\mu$ m)	Germination (%)	Pollen Tube Length ( $\mu$ m)
100	-	-	7	56	15	85
200	9	70	21	96	26	105
300	19	106	10	70	48	274
500	30	254	-	-	27	142
600	22	134	-	-	9	64

**Table 7: Stigma receptivity and in vivo pollen germination of *Solanum sisymbriifolium***

Period after flower opening	24 hrs.	48 hrs.	72 hrs.	Drooping stage
No. of stigmas observed	10	10	10	10
No. of stigmas showing germination	7	5	2	1
Stigma receptivity (%)	70	50	20	10
Mean No. of pollen retained on stigmas	402	455	356	215
Mean No. of germinated pollen	364	237	145	24
<i>In vivo</i> pollen germination (%)	90.54	52	40.73	11.16
Mean pollen tube length ( $\mu$ m)	1406	994	786	204



Fig.6: Thrips on flower



Fig.7: *In vitro* pollen germination (LM-40X)



Fig. 8: *In vivo* pollen germination through pistil (LM-100X)

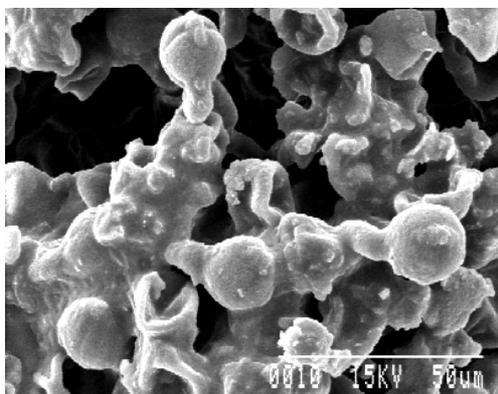


Fig. 9: Pollen tube growth through pistil tissue (SEM – 1000X)

## DISCUSSION

The actinomorphic, wheel-shaped flowers of *S. sisymbriifolium* open early in the morning after that pollen presentation time starts. Flower and pollen anthesis may depend upon physical, physiological and biochemical factors of a plant as well as on climate conditions. Among the different flower visitors (Table 2) the carpenter bees (*Xylocopa* sp.) and *Amegilla* sp. are the most effective and reliable pollinators to this plant. They use “buzz pollination” technique for collecting pollen from the poricidal anthers. The biological potentiality of individual flowers in an inflorescence could be calculated by counting the total pollen grains per flower, but it is very difficult to estimate absolute pollen production (Mandal and Chanda, 1981; Mandal and Ray, 1984). However, pollen per flower and per ovule is related to fertilization rate. In *S. sisymbriifolium* a higher number of pollen per flower, as well as per ovule (Table 1), indicates its xenogamous nature which is genetically superior. The maximum germination (90%) along with pollen tube growth (1305 $\mu$ m) occurs in 20% sucrose supplemented with 100ppm boric acid solution (Table 5, Fig. 7). This is attributed to the fact that sucrose is necessary for proper pollen nutrition, osmotic control and in combination with boric acid promoted pollen germination because boron makes a complex with sucrose which may be easily translocable rather than sucrose alone. The role of boron has been confirmed in germinating pollen and growing pollen tubes (Sidhu and Malik, 1986). Boron may enhance the sucrose uptake and stimulate germinating ability. This observation gets support from Pal *et al.* (1989), Gupta *et al.* (1989), Mandal *et al.* (1997, 1982), Bhattacharya *et al.* (1997), Bhattacharya and Mandal *et al.* (1999, 2000, 2004), Mohi-ud-din *et al.* (2007), Biswas *et al.* (2008, 2009). Stigma remains more receptive during the first day after anthesis (Table 7) (Fig. 8,9). Generally receptivity reaches a maximum soon after anthesis (Shivanna and Johri, 1989) but the period of receptivity may vary from species to species (Joshirao and Saoji, 1989). The present observation gets support Shivanna and Owens (1989), Nepi and Pacini (1993), Ciampolini *et al.* (1996), Tandon *et al.* (2001), Mohi-ud-din *et al.* (2007), Choudhury *et al.* (2008), Biswas *et al.* (2009), Luo *et al.* (2009), Sreekala *et al.* (2011), Aswani and Sabu (2012) and Chauhan (2012).

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