Reproductive biology of Solanum sisymbriifolium Lamk. (Solanaceae) in Tripura, North-east India

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ABSTRACT

Solanum sisymbriifolium or sticky nightshade, an annual plant, becomes increasingly abundant from April until late autumn. Flowers are borne on supra axillary cymose and take 12 to 15 days to convert into mature fruits. Opening of flowers started in the morning between 0600-0800h. During 1 day colour of the flower is white and for the subsequent days the colour changes into light purple. Flower longevity was a little more than 3 days - ranged between 72 to 74 hr (mean ± SE = 73.2± 0.58). The maximum (85%) pollen germination along with 110µm tube development was found in 10% sucrose solution supplemented with 100 ppm boric acid. Pollen grain of S. sisymbriifolium is trizonocolporate. The number of pollen produced per flower varies from 8,75,445 - 8,79,450. The percentage of viable pollen using FDA test is 71.20 ±3.360. The mean pollen: ovule ratio is 10819:1. Chromosome counts revealed that S. sisymbriifolium is diploid i.e., 2n=24. Xylocopa, Ceratina, Amegilla are the effective pollinators. Flowers set fruit in bagged condition indicating self-compatible nature of the plant and the emasculated flowers failed to set fruit showing no evidence of obligate apomixis in the species.

Keywords : Reproductive biology, Solanum sisymbriifolium, self-compatibility, breeding system.

Reproductive biology of angiosperms has its focus on phenology, pollination biology, pollen-pistil interaction and breeding systems. These are valuable for basic and applied research, having implications to ecological and evolutionary studies as well as agriculture and conservation biology. The physiology of reproduction in most of the flowering plants is closely under the control of environmental factors (Taiz and Zeiger 2003). The environment in which an organism lives affects its reproductive success (Sedgley and Griffin 1989). Environment exerts considerable influence on flowering, pollen fertility, in vitro pollen germination and fruiting in plants (Shivanna 2003).

Solanum sisymbriifolium Lamk. commonly known as “sticky nightshade” is an important medicinal plant belonging to the family Solanaceae. The plant is grows wild in the state Tripura and it occurs mostly in dry places, as a weed along road sides and waste lands. Various medicinal properties are attributed to it particularly the fruits and flowers are used as analgesic, in the synthesis of corticosteroids and oral contraceptives (Ferro et al. 2005). The ethanol extract of leaves also shows neuro-pharmacological, anti-diarrheal, and cytotoxic activities (Serker et al. 2013). In spite of its excessive use for medicinal purposes and wide distribution, this species is gradually decreasing due to habitat fragmentation and by increasing urbanization and industrialization. So, there is an urgent need for its conservation and it can be achieved by understanding its reproductive biology. Floral biology of Solanum sisymbriifolium have been studied earlier (Biswas et al. 2013). However, reproductive biology of this medicinally important plant has not been studied so far in Tripura and therefore, the present investigation has been undertaken to study the reproductive biology.

MATERIALS AND METHODS

The present study was carried out on ten marked plants of S. sisymbriifolium growing at Tripura University campus in natural conditions. Observations were recorded every day on flowering phenology, pollen biology, number of pollen and ovules/flower and pollen-ovule ratio and breeding system.

Flowering phenology—The number of flowers on ten marked plants were counted periodically throughout the flowering period. Initiation, peak and end of flowering were registered following the procedure after Dafni (1992). Number of open flowers/plant/day was counted. Time of opening of flowers, anther dehiscence, and stigma receptivity was recorded. Stigma receptivity was determined by examining 100 stigmas at different stages of development for stigmatic secretion. Formation of fruits was observed periodically in tagged floral buds on marked plants. The longevity of flowers was observed by the method after Gill et al. (1998).

Pollen production/anther/flower—Mature anthers were crushed in lactophenol-glycerine with aniline blue. A known dilution was placed on the grid and 10 replicate counts were made using a hemocytometer (Barrett 1985). Morphology of pollen was studied after acetylation (Erdtman 1960). The size of the pollen grains (for radio symmetric ones the diameter in the polar view, and for bilateral ones the polar and equatorial axes) was measured in glycerine jelly (Wodehouse 1935) using standard ocular micrometer.

Meiosis—The young flower buds were fixed in 1: 3 acetic alcohol for 24 h and meiotic behaviour was studied by squash technique using 2% acetic carmine (Belling 1926).

Pollen viability—Pollen viability was checked by mounting pollen grains in fluorescein diacetate (FDA) solution following...
the method of Heslop-Harrison (1970). In vitro pollen germination was also carried out in 2%, 5%, 10%, 15%, and 20% sucrose solutions alone and in these sucrose solutions supplemented with 100 ppm boric acid and different salts like CaCO₃, MgSO₄ (100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm).

**Number of ovules/flower**—Number of ovules/flower was recorded in the pistils fixed in formalin-acetic-alcohol by the procedure described by Shivanna & Rangaswamy (1993). The number of ovules from the cleared ovaries were counted using stereomicroscope.

**Pollination and Breeding system**—60 floral buds were tagged on each plant before anthesis and left for open pollination. 60 flowers/plant were emasculated and bagged. These at the time of stigma receptivity were pollinated with pollen collected from freshly dehisced anthers of the same plant or from flowers of different plants. The number of fruits formed in each hand pollination experiment was recorded.

### RESULTS AND DISCUSSION

**Floral biology**—Flowers are, 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, stout, slightly swollen at the base, whitish, anther more or less oblong, 2-celled, basifixed, dehiscence by apical pores, yellow; carpels-2, syncarpous; ovary, style long, slender, stigma capitulate, bifid, apart from the anthers; ovary 2 chambered with many ovules in each chamber; fruit a berry, bright red, 1-2 cm across, globose, covered by enlarged and reflexed calyx; seeds many, reniform, 2 mm in diameter.

**Flowering phenology**—Flowers open in the morning between 0600 hrs-0800 hrs after that pollen presentation time starts and have yellow coloured oblong anther which are dehisced by apical pores. The stigmas are however receptive a day before anthesis.

**Pollen production**—The number of pollen produced per anther varies between 1,75,490±4,005 and per flower varies from 8,75,445-8,79,450 (Mean: 8,77,448). (Table 1).

<table>
<thead>
<tr>
<th>Floral attribute</th>
<th>Value (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of inflorescence/plant</td>
<td>7.2±0.06</td>
</tr>
<tr>
<td>Number of flowers in an inflorescence</td>
<td>3.2±0.12</td>
</tr>
<tr>
<td>No. of pollen produced/flower</td>
<td>8,75,445±4,500</td>
</tr>
<tr>
<td>Number of fruits/plant</td>
<td>17±1</td>
</tr>
<tr>
<td>Number of seeds/fruit</td>
<td>74±6.18</td>
</tr>
</tbody>
</table>

**Pollen viability**—The percentage of viable pollen as tested by FDA test is 71.20±3.360.

**Meiotic study**—The pollen mother cells undergo normal meiosis producing viable pollen grains. There are 12 bivalents at metaphase I indicating that it is diploid with 2n=24 (Fig. 5). Different chromosomal stages were observed during meiotic division. In Solanum sisymbriifolium, the normal meiosis is indicating the normal pollen development.

**In vitro pollen germination**—The effect of sucrose on in vitro pollen germination of S. sisymbriifolium showed that the taxa required comparatively low sucrose concentration (10%) for their optimal germination (Fig. 1) and to some extent boric acid also influences the percentage of pollen germination. But, the best result was obtained in 10% sucrose solution supplemented with 100 ppm boric acid (Fig. 2). It was also observed that 300 ppm of CaCO₃ showed highest pollen germination (76%) along with longer pollen tubes whereas 100ppm of MgSO₄ (72%) showed good result (Figs. 3 & 4). It has been observed that concentrations of CaCO₃ higher than 300ppm were toxic and they showed minimum germination percentage. Similarly, concentrations of MgSO₄ higher than 100ppm also showed shorter pollen tubes and minimum germination percentage. Boron is known to combine with sugar to make a sugar-borate complex which is translocated with greater facility rather than or less borate sugar molecules and boron may enhance the sucrose uptake and stimulate germinating ability (Shivanna and Johri 1985). Mohi-ud-din et al. (2007) and Biswas et al. (2008) have also recorded similar observations. Cook and Walden (1965) reported that the presence of a calcium ion is required for pollen germination. In the present work germination increased with an increase in concentration of Calcium carbonate but decreases when increased the concentration of MgSO₄ higher than 100ppm.

**Reproductive Success**—The mean pollen: ovule ratio is 10819:1. High P/O ratio along with high pollen production in Solanum sisymbriifolium attributes to its high seed set.

**Pollination biology and breeding system**—Several insects and thrips visited the flowers frequently throughout the day (Figs. 5 A, B, C, D). Among them Apis dorsata, Xylocopa sp., Ceratina sp. Amegilla sp., Mosquito bug, Scarabaeidae beetle continuously visit the flowers and pollen grains adhere to their body parts which they transfer to another receptive stigmas. Xylocopa, Ceratina, Amegilla are the effective pollinators. Thrips also enter the flowers even at bud stage for pollen and transfer pollen on the receptive stigma. A maximum fruit set of 85% was obtained under open pollination. Flowers emasculated (n=60) and bagged to ascertain the occurrence of apomixis and there is no fruit set. Flowers bagged to check for self pollination (n=60) and a maximum of 53.33% fruit set was recorded which indicates self-compatibility. The percentage fruit set through xenogamy was higher than through geitonogamy.
**Fig. 1**— *Solanum sisymbriifolium* Lamk. A-C. Pollinators; D. Thrips; E. Pollen viability; F. In vitro pollen germination in 100ppm boron+10% sucrose solution; G-H. Pollen mother cell showing 12 bivalents at metaphase.

**Fig 2**— Pollen germination & tube length at different concentrations of sucrose.

**Fig 3**— Pollen germination & tube length at different concentrations of sucrose + boric acid.

**Fig 4**— Pollen germination & tube length at different concentrations of CaCO$_3$.

**Fig 5**— Pollen germination & tube length at different concentrations of MgSO$_4$.

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**REFERENCES**


