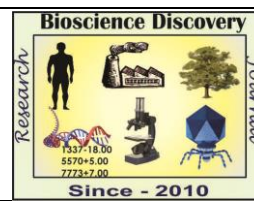


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Research Article



Reproductive biology of *Solanum ovigerum* Dunal

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Abstract

The present article recorded different reproductive parameters of this species including floral morphology, foraging behaviour of flower visitors, pollen morphology, production, viability, germination, pollen ovule ratio, stigma receptivity and the meiotic as well as breeding system. Flowers are extra-axillary and take 20 to 30 days to convert into mature fruits. Flowers open in between 0500-0600h and flower longevity lasts for 3 days ranged between 70 to 72 hr. One flower produces an average of 4, 77,448±2,002 pollen grains. The maximum (51.77±2.33%) pollen germination along with 112µm tube development was found in 5% sucrose solution supplemented with 100 ppm boric acid. Pollen viability was determined using FDA test. The mean pollen: ovule ratio is 663:1. Stigma became receptive between 0650-1400 hr. Chromosome counts revealed that *S. ovigerum* is diploid i.e., n=12. 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.

INTRODUCTION

The study of plant reproductive biology has been illustrated as the congregation of knowledge on the system of both sexual as well as asexual reproduction in plants (Sreekala, 2017). Throughout the centuries the reproductive biology and pollination of plants has been a traditional subject for both professional and put down people of naturalist's attention (Ayasse and Arroyo, 2011). The study 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. For the establishment of successful cultivation and conservation of plants a complete understanding of their reproductive biology is required (Moza and Bhatnagar, 2007).

Solanum ovigerum Dunal (Solanaceae), a prickly shrub which is related to *Solanum melongena*, is generally cultivated throughout

Tripura for its white edible fruits resembling hen's egg and therefore, it is also known as Anda begun by the local people of the state. This plant generally blooms year-round but the peak flowering period is May-June and bears fruits in July-August. The stem and leafstalks are green, or very faintly tinged with purple, and bear white spines. Leaves are wavy at the edges and the flowers are lilac. In most of the villages of Tripura, their fruits are consumed as well as marketed by the ethnic groups and therefore considered as an important crop of subsistence especially in tribal areas of Tripura. According to the tribal community, various medicinal properties are attributed to it, particularly the fruit helps to lower blood cholesterol levels and is suitable as a part of their diet to help regulate high blood pressure. The ashes of the peduncle are used in the treatment of intestinal haemorrhages, piles and toothache. A decoction of the root is astringent. The leaves are narcotic and a decoction is applied in clearing wounds and internal haemorrhages.

However, reproductive biology of this important plant has not been studied so far in North-East India as well as in India and therefore, the present paper is a part of the attempt to made to understand the reproductive biology of *Solanum ovigerum*.

MATERIALS AND METHODS

The present study was carried out in Tripura University campus area. Ten healthy plants were selected and observations were made on a day-to-day basis in natural habitats on flowering phenology which includes habit, phenology, anthesis etc. Floral morphology was studied with the help of hand lens and dissection microscopy. *In vitro* pollen germination was carried out in different concentration of sucrose (2%, 4%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35% and 40%) alone and in combination with 100ppm boric acid (Brewbaker and Majumder, 1961). Pollen morphology has been studied following Acetolysis methodology as proposed by Erdtman (1960). The pollen production study was done following the standard methods (Nair and Rastogi, 1963; Mandal and Chanda, 1981). The pollen viability was assessed using Fluorescein Diacetate (Heslop-Harrison and Heslop-Harrison, 1970). The pollen ovule ratio was determined following the method of Cruden (1977). Stigma receptivity was observed by fixing stigmas of varying ages at different time intervals in Carnoy's fixative for 3 – 4 hrs., then stained with aniline blue-lactophenol and observed under microscope (Hauser and Morrison, 1964). Meiotic behaviours of chromosomes have been studied by Acetic carmine smear technique (Belling, 1926). For pollination and breeding system, 100 floral buds were tagged on each plant before anthesis and left for open pollination. 100 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

Phenology: *Solanum ovigerum* or Anda Begun is widely cultivated for its edible fruits. Initiation of floral buds takes approximately 26 days to develop from vegetative shoots where as floral buds take 8 to 10 days to bloom. Flowers take 20 to 30 days to convert into mature fruits. Opening of flowers

started in the morning between 0500-0600h after that pollen presentation time starts and have yellow coloured oblong anther which are dehisced by apical pores. Stigma became receptive between 0650-1400 hr. Flower longevity was for 3 days ranged between 70 to 72 hr (mean \pm SEM = 71 \pm 0.45). Flowering period continues throughout the year.

Phenology is the study of periodicity in the life-cycle events of plants and animals which are influenced by seasonal variation in weather factors. Reproductive phenology or the timing of the gradual series of events for flowering and fruiting is a crucial determinant of reproductive success and offspring's genetic diversity in plant species (Rathcke and Lacey, 1985; Fox, 2003; Weis and Kossler, 2004; Elzinga *et al.*, 2007). Overlap in flowering time among individuals will determine the mating opportunities available and will constrain the genetic diversity within the progeny (Loveless and Hamrick, 1984; Lyons and Mully, 1992; White and Boshier, 2000; Bawa *et al.*, 2003; Kitamoto *et al.*, 2006). Flowering phenology, coupled with plant density and spatial structure, will influence pollinator visitation, gene flow, and ultimately fruit production (Rathcke and Lacey, 1985; Marquis, 1988; Ollerton and Lack, 1998; Bustamante and Burquez, 2008). *S. ovigerum* has long term flowering period i.e., continuous flowering almost throughout the year. So, the constant production of flowers and fruits observed contributes to the maintenance of pollinators that use resources provided by the species. Thus flowering phenology is an essential component in understanding the reproductive ecology of perennial plants.

Floral biology: Flowers are extra-axillary, pedicellate, complete, actinomorphic, bisexual, hypogynous, lilac; sepals-3-5, polysepalous, spiny, persistent, green; petals-3-5, gamopetalous, purple, sometimes recurved; stamens-3-5, filament short, stout, slightly swollen at the base, whitish, anther oblong, 2-celled, basifixed, dehisces by apical pores, yellowish; carpels-2, syncarpous; style long, medium, short as well as rudimentary, stigma green, wet, lobed (0.5-1.0 mm), apart from the anthers; ovary 2 chambered with many ovules in each chamber; fruit a berry with a persistent calyx are very white when immature, but turn yellow when ripe. In shape and size, they greatly resemble an ordinary hen's egg (**Plate 1**); seeds numerous, compressed, brown in colour.

Table 1: Values of different floral attributes of *S. ovigerum*

Floral attribute	Value (Mean±SEM)
Number of inflorescence/plant	8.4 ± 0.68
Number of flowers in an inflorescence	3.8 ± 0.38
No. of pollen produced/flower	4,77,448±2,002
Number of fruits/plant	8.4 ± 0.51
Number of seeds/fruit	719.9 ± 35.22

Floral visitors: A total of 5 genera of floral visitors were recorded. Several insects and thrips visited the flowers frequently throughout the day. Among them *Apis dorsata*, *Xylocopa* sp., *Ceratina* sp. *Amegilla* sp. continuously visit the flowers and pollen grains adhere to their body parts which they transfer to another receptive stigmas. The floral visitors' activity was less in the afternoon, however, from 16.30 hrs onwards and towards the evening hours activity was again more. *Xylocopa* sp. and *Apis dorsata* visited the flowers regularly throughout the day time whereas Trips visited in day as well as at night. *Apis dorsata* visited one to seven flowers in a single spell. Duration of the visit was 5 to 20 seconds per flower. Bees visited one to five flowers in a single spell and the duration was four to sixty seconds per flower. *Apis dorsata* visited the flowers to collect pollen i.e. they help in buzz pollination which was found to be most valuable process in *Solanum* species. Their frequent visit from one flower to other may perhaps help transfer of pollen. The activity of floral visitors starts after the opening of the flowers. The insect activity diminishes in cloudy days.

Pollen morphology: Pollen grains are isopolar, symmetrical and trizonocolporate. Polar axis P (26.24-) 26.88 ± 1.35 (-27.52) μm and Equatorial diameter E (25.26) 25.69 ± 0.47 (-26.12) μm . P/E:1.05. Exine (2.28-) 2.73 ± 0.76 (-3.18) μm thick. Lashin (2011) has made comparative investigation of six species of *Solanum* from Saudi Arabia and recommended that the pollen morphology is isopolar, symmetrical, trizonocolporate and the size of species under concern showed overlapping.

Pollen viability: The percentage of viable pollen as tested by FDA test is 71.5 ± 0.86 . According to Dafni and Firmage (2000) pollen viability is considered as an important parameter of pollen quality. Pollen viability refers to the ability of complete the post pollination events on a compatible, receptive stigma and effective fertilization. The term 'pollen viability' should be used carefully and rather replaced by the more limited term 'pollen

stainability', as it depends strictly on the staining assay (Bhowmik and Datta, 2012).

Pollen production: **The number of pollen produced per anther varies between 1,75,490±2,002 and per flower varies from 4,75,445-4,79,450 (Mean: 4,77,448) (Table 1).** With regard to pollen production, the present work relates with the studies on pollen production in order to locate the differences in the biological potential of individual flower in an inflorescence (Nair and Kapoor, 1973). The pollen production is a characteristic of all plants, and is by definition, an integral part of the pollination process (Haynes, 1988).

In vitro pollen germination: The effect of sucrose on *in vitro* pollen germination of *Solanum ovigerum* showed that the studied taxa required very low sucrose concentration (i.e. 2%, 4%, 5% and 8%) for their optimal pollen germination and boric acid to certain extent also influence the percentage of pollen germination. The taxa showed optimal pollen germination at 5% ($50.44 \pm 1.16\%$) sucrose solution but the best result i.e. $51.77 \pm 2.33\%$ (**Fig 2, Plate 1**) and a maximum tube length of 112 μm was obtained from 5% sucrose and 100 ppm boric acid in combination. This is attributed to the fact that sucrose is necessary for proper pollen nutrition, osmotic control and in combination with boric acid promotes pollen germination (Sidhu and Malik, 1986). Boron combines with sugar and form a sugar-borate complex which is translocated with greater facility than sugar molecules without boric acid (Gauch and Duggar, 1953). Boron may enhance the sucrose uptake and also stimulate germinating ability which has been confirmed by the study of other workers (Pal *et al.*, 1989; Gupta *et al.*, 1989; Mandal *et al.*, 1982; Bhattacharya *et al.*, 1997; Mohi-ud-din *et al.*, 2007; Biswas *et al.*, 2008). According to Pfahler *et al.* (1997), *in vitro* pollen germination and pollen tube growth are very useful for explaining the lack of fertility. Pollen germination and the growth of pollen tubes are important and necessary for proper fertilization and seed formation in flowering plants.

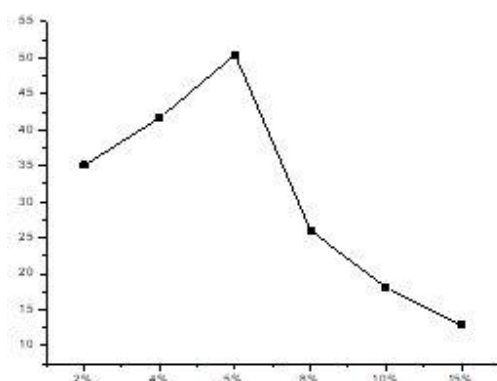


Fig 1: Pollen germination at different concentrations of sucrose.

Pollen ovule ratio: The mean pollen: ovule ratio is 663:1. The pollen ovule ratio is an important floral trait that reflects the mating system of a plant (Cruden, 1977; Preston, 1986). According to Shivanna and Johri (1985), rather than the total pollen per flower or per plant ratio, the pollen-ovule ratio is more accurate measures of reproductive success. The data of pollen: ovule ratio of *S. ovigerum*, ratified with the result reported by Cruden, indicates that the species to be facultatively xenogamous.

Stigma receptivity: The ability of the stigma to support pollen germination and tube growth of viable compatible pollen grain is referred as stigma receptivity (Shivanna, 1998). According to Joshirao and Saoji (1989) for successful completion of the post-pollination events receptivity of the stigma is a critical factor. It is a critical stage during maturation of flower which highly influences the success of pollination in the life cycle of flowers (Barrett, 2002).

The stigma of *S. ovigerum* became receptive between 0650-1400 hr. During receptive state, stigmas remain lustrous and greenish and after losing the receptivity it becomes brownish. The period of receptivity is influenced by environmental factors such as temperature and humidity. During the cloudy and rainy days normally receptive period of stigma is extends up to the second day of flowering.

Meiotic study: The chromosome number of *Solanum ovigerum* is counted to $n = 12$, i.e. with 12 bivalents. Different chromosomal stages were observed during meiotic division. In *Solanum ovigerum*, the normal pollen meiosis (Plate 1, g) is indicating the normal pollen development, however, in some cases though the frequency is very low (2-

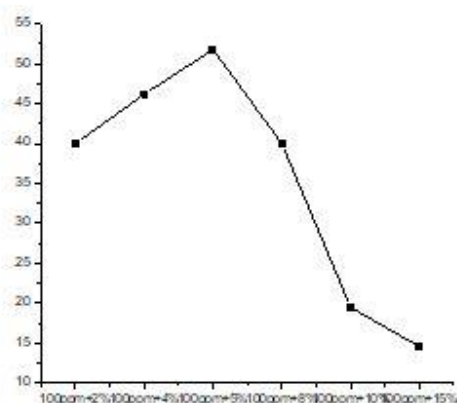


Fig 2: Pollen germination at different concentrations of sucrose + 100 ppm boric acid.

3%) they show some meiotic abnormalities (Plate 1, h, i, j, k) such as sticky bridge, laggard chromosome, abnormal separation and irregular distribution of chromosome during telophase. Meiotic irregularities were found in some plants which may be interpreted as a sign of disharmony in the genetic systems of the individuals. The amount of genetic variability in the gametes may be increased by such type of structural changes in chromosomes by forming new genetic linkage groups that may be used for adaptation to adverse environmental conditions (Talukdar, 2009). High levels of meiotic irregularities compromise pollen viability (Bala and Gupta, 2014) and low pollen grain viability could be either associated to the meiotic irregularities, or to post-meiotic events (Ferreira *et al.*, 2009) which may lead to the formation of sterile seeds. However, the species showed high percentage of pollen viability so these less frequent meiotic abnormalities observed in the present study does not affect the fertility of pollen grains in the population.

Breeding system: A maximum fruit set of 67% was obtained under open pollination. Flowers emasculated ($n=100$) and bagged to ascertain the occurrence of apomixis and there is no fruit set. Flowers bagged to check for self pollination ($n=100$) and a maximum of 42% fruit set was recorded which indicates self-compatibility. The percentage fruit set through xenogamy was higher than through geitonogamy.

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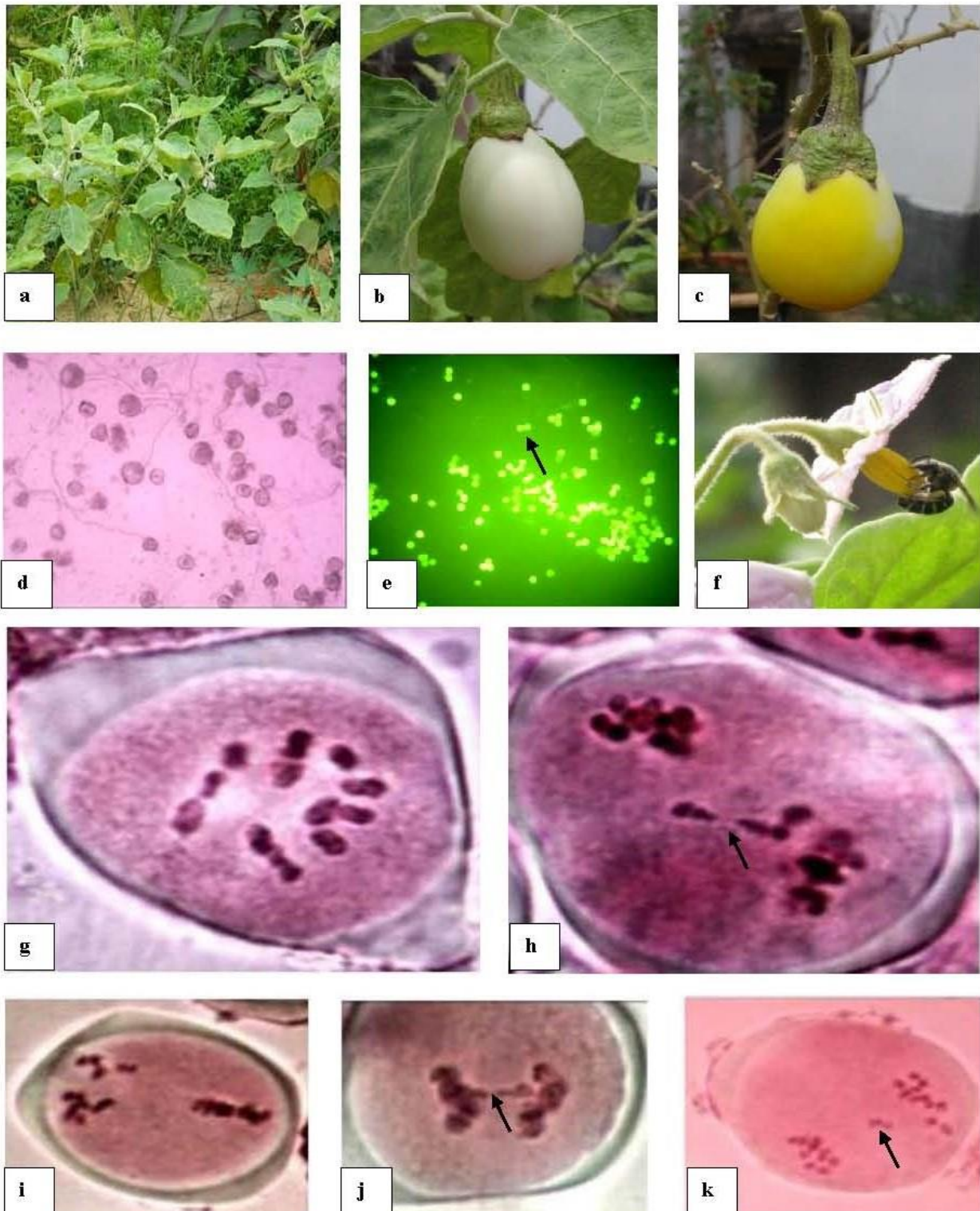


Plate 1: *Solanum ovigerum* a) Habitat; b) Young fruit; c) Mature fruit; d) 5% sucrose solution + 100 ppm boric acid; e) Viability using FDA (Arrow showing viable pollens); f) *Apts dorsata* (Pollinator); g) Pollen Mother Cell of Metaphase I showing 12 bivalents; (h & k) chromosomal laggards; (i) Unequal polarity; (j) chromosomal bridges.

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