Universal Impact Factor 0.9285:2012; 1.2210:2013

Index Copernicus
ICV 2011: 5.09
ICV 2012: 6.42

NAAS Rating
2012 : 1.3;2013:2.69

Received on:
14th March 2014

Revised on:
19th March 2014

Accepted on:
20th March 2014

Published on:
1st June 2014

Volume No.
Online & Print
52 (2014)

Page No.
71 to 78

REPRODUCTIVE BIOLOGY OF THE TROPICAL VULNERABLE SHRUB *CAPPARIS SHEVAROYENSIS* SUND.-RAGH. (CAPPARACEAE)

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ABSTRACT:

Capparis shevaroyensis Sund.-Ragh. (Capparaceae) a vulnerable shrub was studied for its phenology, flower morphology, pollen viability, stigma receptivity and seed setting. This species was found distributed in a narrow range in the Karanadamalai hills and Sirumalai hills of Eastern Ghats of Tamilnadu, India. The study reveals that it has very poor pollen germination and subsequent limited seed setting. It produces enormous number of pollens (953/anther) where all of them were viable in X-gal test. Number of fruits per flower was limited to one per inflorescence and many of the seed were found infected by insects. It has very limited population, owing to its habitat condition besides its reproductive features which further move the species to endangered condition.

KEY WORDS: *Reproductive biology, Tropical, Vulnerable, Shrub, Capparis shevaroyensis, Capparaceae.*

INTRODUCTION:

Many factors affect the reproductive success of flowering plants. Among these factors, the timing, frequency, and duration of the flowering period (collectively referred to here as phenology) are obviously of great importance (Rathcke & Lacey, 1985). The phenology of a species not only encompasses when, how often, and how long reproduction takes place but also determines

the degree of reproductive synchrony with other plant species (Rathcke, 1988a, b).

Despite seasonality, there is substantial overlap in the timing and duration of flowering among plant species (Rathcke, 1988a, b; Gross *et al.*, 2000). However, there are examples of phenological patterns that result in little or no overlap with other species or result in reproduction during climatically unfavorable periods. For example, in neotropical dry forests, many trees flower during the dry period (Bullock & Solis-Magallanes, 1990) rather than the perhaps climatically more favorable growing season.

Capparis L. is one of the genera having high percentage of endemism in India, while 9 species are confined to India, 5 are endemic to India and Sir Lanka (Sharma & Balakrishna, 1993). Most tropical woody plants produce new leaves and flowers in bursts rather than continuously and most tropical forest communities display seasonal variation in the presence of new leaves, flowers, and fruits. This patterning suggests that phenological changes represent adaptations to either biotic or abiotic factors. Biotic factors may select for either a staggering or a clustering of the phenological activity of individual plant species (Van Schaik *et al.*, 1993). Tropical tree development should be arrested during drought and proceed actively during periods of adequate rainfall. In many tropical deciduous forests, the major phenological events confirm to these expectations (Frankie *et al*., 1974).

The aim of this paper is to describe the phenology, pollen counting, pollen viability, pollen germination and stigma receptivity of *Capparis shevaroyensis* a vulnerable shrub that are distributed in Eastern Ghats of Tamilnadu.

METHODOLOGY:

Reproductive Biology

1. **Phenology:** Information on the date and month of flowering, fruiting, shedding of leaves and spring of new leaves were collected from the Flora of Tamilnadu Carnatic (Matthew, 1983) and Flora of Palni hills (Matthew, 1998). Besides, this information also verified from the field survey and affirmed and taken record in the field notes (Sharma *et al.*, 2011).
2. **Information on Inflorescence:** Ten inflorescences at random from different individuals were sampled for the number of flowers per each inflorescence.
3. **Flower morphology:** Fresh flowers were collected and preserved in 5% formaldehyde solution for further study. Features such as the size of flower, number of bracts, sepals, petals, filaments, anthers, styles, carpels and ovules were counted and recorded (Koning, 1994; Banu *et al.*, 2009).
4. **Number of anther/flower:** Information on the number of anther per flower and number of anther lobe per filament were counted and recorded.
5. **Number of carpel, ovule/flower:** Transverse and longitudinal section of ovary were prepared and the number of carpel and ovule per carpel were counted and recorded.

6. **Time of opening of flower:** In the field the opening time of flower was recorded in the observation note book.
7. **Number of pollen/anther:**

   Anthers are stored in 0.5 ml of ethanol in an eppendorf tube to release the pollen grain (Kearns & Inouye, 1993). The anthers must be transferred into 1 normal hydrochloric acid the day before; the anthers will sit in the HCl overnight. Place 0.5 ml of 3:1 lactic acid, glycerin solution into the tube of the tissue homogenizer. Remove the anthers from the HCl vial and place them in the tissue homogenizer without transferring any of the HCl.

   The next step is to place the small part of the homogenizer into the larger tube of the homogenizer to break the anthers. Crush the anthers so that there are little to no remnants of the anthers remaining. Place one drop on each section of the haemocytometer and cover the solution with a cover slip making sure that only one cover slip is used (more than 1 cover slip will make it hard to count the pollen because the lines of the haemocytometer will not be visible through the microscope).

   Place the haemocytometer on the microscope. The key to seeing the lines of the haemocytometer is to adjust the contrast. Once the lines of the haemocytometer are visible, counting can begin. Counting the pollen is done by counting the pollen grains in each small box that makes up 1 big box. 16 small boxes (4 by 4) make 1 large box. Once all of the small boxes that make up 1 large box are counted the slide can be moved to view a different large box and the pollen must be counted in that one as well. This should be repeated for about ten large boxes.

   Make sure a box is not counted twice; a good way to prevent this from happening is to count the small boxes in order from left to right then move down a row and count from right to left and repeating this until all 16 small boxes are counted. Furthermore, if a pollen grain is on the outer line of the large box, only count it if half of it or more is inside the box. Once the counting is completed an average of the pollen grains must be calculated. This is the average pollen grain count per large box. This is them multiplied by 2500 to find the average pollen count per flower. All data in the experiment were subjected to analysis of variance and the mean separation was done by Turkey's MRT at $P \leq 0.01$.
8. **Pollen viability:** Pollen has a very important role in the flow of genes in plants, especially in plants that are out crossing. The first method for testing pollen involves determining viability by staining. The X-gal test to determine the content of β-galactosidase (an enzyme involved in the lactose degradation). The X-gal test consists of a solution of 1 mg Xgal (5-bromo-4-chloro-3-indoyle-β-galactoside) that is dissolved in 50 µL N,N-dimethyl formamide and 1 mL acetate buffer (50 mmol with pH 4.8). Viable pollen turns blue (Wang *et al.*, 2012).

9. **Pollen tube germination:** In the second method for testing the viability, germination tests were carried out to measure pollen viability. There are two major tests, which can be divided in two different parts. *In vitro* germination, pollen is grown on a specific media. *In vivo* germination pollen is grown on the stigma of the plant. In the present study the *in vitro* germination was conducted.

***In vitro* germination for pollen viability**

Fresh harvested pollen is grown on a medium containing 1% agar, 20% sucrose, 0.01% boric acid and 0.01% calcium nitrate. These compounds have been shown to be very important for pollen germination in different species. The pollen is grown in a humid environment and at room temperature (~20°C) for 8 hrs. The pollen is considered mature when the pollen tube length is longer than the diameter of the pollen grain. Germination was scored by a light microscope (x 100) in four random fields (about 50 grains / field) (Wang *et al*., 2004).

10. **Stigma receptivity** Baker's procedure (Dafni, 1992):

This test detects the presence of alcohol dehydrogenase. The test solution consists of 10 ml of 1 M phosphate buffer (pH 7.3–7.5), diluted (1 part buffer to 2 parts distilled water); 5–10 mg nitroblue-tetrazolium to give a slight yellow colour; 6 mg of nicotinamide adenine dinucleotide; and 1 ml of ethanol (95%). The fresh stigma was cut and removed in the field directly into a large droplet of this test solution on a slide and incubated at room temperature in a closed petri dish containing a moist filter paper in the bottom. The stigmas were inspected after 20–40 min under a magnifier (×20) or a microscope (×200) to locate the stained areas.

11. **Fruit and seed characters**: The weight of fruit and seed were measured and the number of seed per fruit was also calculated.

RESULTS AND DISCUSSION:

Capparis shevaroyensis is collected from Karanthamalai hills (10°17′29" N and 78°13′44" E), part of the Eastern Ghats of Tamilnadu in the month of August 2012 and October 2012. Voucher collections for each were made and deposited in the herbarium of the Department of Botany, St. Joseph's College, Tiruchirappalli.

Flowering period

Flowering of *Capparis shevaroyensis* was observed in the month of March-April and August-September while fruiting was observed in the month of July-August and November-December. On a random subset of nine flowering individuals were monitored to observe the time flowering. The flowering time was noted 10.00hr to 13.00hr in the month of 28 August 2012. The phenomenon of flowering and fruiting is determined by the photoperiod of a specific region which again is influenced by the variation in seasons such as monsoon, winter and summer, hence always differs from one geographical region to other.

Information on Inflorescence

Umbellate inflorescence was noticed at the base of foliage axillary. Each inflorescence consists of four to eight flowers and an average of six flowers.

Flower Morphology

Flowers white, in 5 – 10 flowered, sub terminal and axillary umbels; peduncles 0.5 - 3 cm long; pedicels 1 – 1.2 cm long, glabrous. Sepals 4, ovate – oblong to obovate, 3 – 6 x 2 – 4 mm, concave. Petals 4, white, oblong – elliptic, 5 – 7 x 2 – 4 mm, sericeous tomentose on both sides, ciliate at margin. Stamens 20 – 27, glabrous; filaments curved, 20 in number, 0.6 – 1 cm long; anthers oblong, 1 – 2 mm long. Style reduced; Gynophore 0.5 – 1.2 cm, purplish. Ovary ovoid, 0.1 – 0.2 x 0.1 cm; style minute, bristle like; stigma obscure. Drupes globose, 1 – 1.2 cm across; seed solitary, ovoid - globose, 6 – 8 mm across, furrowed, blackish; Parietal placentation. Identification of the functional and adaptive significance of variation in flower morphology is fundamental to our understanding of the processes that shape patterns of seed production and floral evolution (Campbell, 1991; Schemske & Agren, 1995; Galen & Cuba, 2001; Aigner, 2004). The position of the stigma within the flower is a key aspect of flower morphology which influences the efficiency of pollen transfer (Campbell *et al.*, 1996; Cresswell, 2000; Nishihiro *et al*., 2000).

Number of Ovule/Flower

This species had one to two ovules in a carpel.

Number of Pollen/Anther counting

The number of pollen ranged from 875 to 1044, mean number of pollen being 953.

Pollen viability

The study revealed that the range of pollen fertility in 100 percentage indicating that this factor does not influence the seed setting behaviour (Figure 1). There could be factors other than pollen fertility influencing seed setting percentage. The pre-fertilization stages like pollen germination, pollen tube elongation might be sensitive to lower minimum temperature resulting reduced seed set. Similar observations on higher pollen fertility and lower spikelet fertility were recorded in rice by Sampath (1964), Balaravi (1967) and Sivasubramaniam *et al*., (1972).

***In vitro* germination for Pollen viability**

Pollen did not germinate at room temperature but pollen viability is 100%. Interestingly pollen fertility percentage had no association with seed set percentage, number of seeds per panicle and grain yield per plant in all the dates except 3rd date. This indicates that pollen fertility may not be related to spikelet fertility. Previous reports have indicated higher pollen fertility but lower seed set (Sampath, 1964). This suggested that the two aspects of sterility *i.e.*, pollen sterility and spikelet sterility may have distinct

causes. It is also possible that, environmental factors may influence at pollen germination and pollen tube growth stages and not at the pollen production level (Mukri *et al*., 2010).

Stigma receptivity

The stigma was light yellow in color. After treated the color of the stigma has changed to dark pink in color. Stigma position is a key aspect of flower morphology that may influence pollination success and seed production. In numerous plant species stigmatic receptivity decreases as the flower ages. At senescence, the stigmatic papillae in *Actinidia* lost their integrity, cellular content was released into the stigmatic fluid, and the secretion contained phenolic compounds which may regulate whether pollen germination occurs (González *et al*., 1994; 1995).

Fruit characters and Seed setting

In this species each inflorescence had 4-8 flowers, randomly unripe fruit per inflorescence was three and finely the fruit setting reduced to one (very rarely two). Fifty fruits and seeds were weighed and the average fruit and seed weight were 0.257 g and 0.135 g respectively. The number of seeds per fruit varied from one to two (six fruits had 2 seed and 44 fruits had 1 seed)**.**

ACKNOWLEDGEMENTS

The authors acknowledge the generous fund provided by Department of Science and Technology (DST) under Fast Track Young Scientist program.

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