



Floral and Pollination biology of Asian spider flower (*Cleome viscosa* L.)

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ABSTRACT

Cleome viscosa grows wild in the fields in different parts of northern-India during the rainy season (July-October). It is an annual herb and the flowers, borne on typical raceme are yellow, complete, hermaphrodite, actinomorphic and hypogynous. They open daily during 0330-0500 h and offer both pollen and nectar as floral resource. Foragers include honey bees, butterflies, flies and ants. Honey bees, butterflies and flies affect autogamy, geitonogamy and xenogamy, while ants affect only auto- and geitonogamy. This species shows facultative xenogamy, but mostly eliminates forming fruits from self-pollinated flowers.

Keywords : *Cleome viscosa*, Cleomeaceae, Asian spider plant, floral biology, pollination, breeding system.

Cleome is a genus of flowering plants earlier placed in the family Capparaceae and later in Cleomeaceae and now in Brassicaceae. DNA analysis indicated that the genera of the family Cleomeaceae are more closely related to Brassicaceae (Stevens 2001). The genus *Cleome* includes about 275 species of herbaceous annual or perennial herbs and shrubs (Huxley 1992). Species of *Cleome* are commonly known as Asian spider flowers, spider plant, spider weed, or bee plant (Steve and O'Kane 2011, Grubben 2004). One of the common species, *Cleome viscosa* is known as the Asian spider flower or tick weed. It is an annual herb commonly found in rainy season. Both seeds and leaves are medicinally used. Due to rapid urbanization, industrialization and its medicinal importance, this plant species is being ruthlessly destroyed and is on the verge of extinction.

The loss of world's biological diversity, mainly due to habitat destruction, over harvesting, pollution and inappropriate introduction of foreign plants continues unabated. Efforts are needed to conserve and maintain genes, species and ecosystem with a view for sustainable management and use of biological resources. Protection of ecosystem and *in situ* and *ex situ* conservation can help to save endangered plants species. This requires a detailed knowledge of the reproductive biology of the species to be conserved (Moza and Bhatnagar 2007, Chauhan and Chauhan 2013). Chauhan *et al.* (2007) have earlier studied a few aspects of the reproductive biology of *C. viscosa*. The present study has been undertaken to investigate the floral biology and pollination mechanism in *C. viscosa*, an endangered species, growing at different sites of Agra city.

MATERIAL AND METHODS

The present investigation was carried out on twenty five marked plants of *C. viscosa* growing in different parts of Agra. Observations were made on the timings of recently opened flowers, number of first-day flowers, flowering and fruiting events. Thirty flowers from selected individuals were used to record the floral morphometry following the method

described by Kearns and Inouye (1993). Morphology of floral parts and pollen grain was also studied by scanning electron microscope (SEM). For SEM analysis, fresh flowers were fixed in 3% glutaraldehyde in 0.1M phosphate buffer, dehydrated through aqueous acetone series, dried with CO₂ in a HCP-2 Hitachi Critical point dryer using liquid CO₂ at 1000 lbs per inch. The samples were mounted on stubs, coated with gold (20 nm) and stored in desiccators. They were observed and photographed using Philips EM 501 SEM at All India Institute of Medical Sciences, New Delhi.

The time of daily anthesis and anther dehiscence was recorded. Number of pollen grains/anther/flower was determined from 50 begged flowers following Kearns and Inouye (1993). Pollen size was measured under a light microscope following the method by McKone and Webb (1988). Stigma receptivity was determined by placing fresh pollen on stigma and periodically observed for pollen germination under a microscope. Pollen-ovule ratio (number of pollen grains divided by the number of ovules) per flower was yielded according to procedure by Cruden (1977). The pollen viability was assessed by both *in vitro* and *in vivo* germination studies. *In vitro* pollen germination was studied by the hanging drop method according to Brewbaker and Kwack (1963). *In vivo* germination was studied by aniline blue fluorescence microscopic method as described by Shivanna and Rangaswamy (1992). Nectar was squeezed into a micropipette in order to measure the volume of nectar (μ l) per begged flower (n=50). Sugar separation was accomplished using paper chromatography following Harborne (1973). Breeding behaviour by autogamy (bagged and hand pollinated), geitonogamy and xenogamy was tested by controlled pollination studies. In order to observe the rate of natural fruit-set, fifty inflorescences from 50 different plants were tagged and were followed until fruit development. Further, observations on the duration of capsule maturation, dehiscence and seed dispersal mode were made. Pollination efficiency of different insects was assessed by observing the

pollen load on different body parts under light microscope according to procedure given by Kearns and Inouye (1993).

RESULTS AND DISCUSSION

Habit : *Cleome viscosa* is an annual and viscid herb. Plants are erect, branched and 60±30 cm tall (Fig. 1a). Leaves are 4±2 cm long and 4±3 cm broad. with 30±15 cm long petiole. Ovate, pentafoliate, leaflets sub-sessile, 2.1±1.4 x 1.5±1.0 cm, elliptic-oblong or obovate, margins entire, apex obtuse, surface glandular-hirsute (Fig. 1a, b).

Phenology: Its full vegetative growth occur following monsoonal rains in June and it flowers from the last week of July until second week of October with the flowering peak in August (Fig. 1a, b). Fruiting starts in the second week of August and the fruits grow to full size over a period of two months (Fig. 1e, f, g).

Floral biology: The inflorescence is a typical raceme and each raceme consists of 9.0±3.0 yellow, complete, pedicellate, hermaphrodite, actinomorphic and hypogynous (Figs. 1a, c). The calyx presents four lanceolate, sepals of 7.5±2.5×1±0.52 mm, and glandular. They are arranged in two sets of two each, green and valvate. The corolla present four petals, two proximate and two spreading, yellow and valvate (Fig. 1a). Stamens are numerous as many as 18.0±2.5 (Fig. 1a). The anthers are dithecous, basifixed and introrse. The pistil is single and differentiates into a capitate and papillate stigma (Fig. 1b), the style is short and the ovary is bicarpellary, superior and unilocular lying on a short gynophore. The ovary contains 25.0±3.5 ovules lying on two parietal placentae. Numerous capitate trichomes are present on the ovarian surface (Fig. 2a). Ovarian trichomes have also been observed in some members of the family Leguminosae e.g. *Cassia occidentalis* (Sharangpani and Shirke 1996) and *Cassia tora* (Sharma *et al.* 2005).

Young fruits are green, linear and covered with numerous capitate trichomes (Fig. 1c). Fruits are capsules similar to siliqua and dehisce partway from apex to base. Mature fruits are 30–100 × 2–4 mm in size and creamish brown (Fig. 1e, f) with large number of seeds which disperse by mature fruits dehiscing from apex to base (Fig. 1f, g). Seeds dark brown resembling snail shells, 1.2–1.8 × 1–1.2 mm, finely ridged transversely.

Flowering phenology : The flowers open daily between 0330 and 0500 h and the anthers dehisce by longitudinal slit around 0400–0530 h. The pollen grains are spherical and tricolpate with reticulate exine (Fig. 2b) and are 23.5 µm in diameter. The number of pollen grains per anther and per flower is 2710±1526 and 48780±13594 respectively. The pollen-ovule is 1951:1. The stigma becomes receptive after the anthers dehiscence around 0700 h and remains so until 1200 h of the same day. *In vitro* pollen germination studies indicate that pollen grains remain viable for 8h after anther dehiscence. Although at the time of anther dehiscence 97% of the pollen

grains are viable. However, there is a substantial reduction in pollen viability afterwards – 87% (2h), 69% (4h), 41% (6h) and 3% (8h). *In vivo* pollen of germination studies after hand pollination of stigma with the stored pollen show 74% of pollen germination after 4h (Fig. 1d), 45% after 6h and only 7% of pollen germination with the pollen that was stored for 8h.. Nectar is secreted at the beginning of anthesis. The fully open flower contains 1.1±0.2 µl nectar. Nectar contains glucose (51%), fructose (26%) and sucrose (23%).

Pollination biology: The flower visitors were insects. The daily foraging schedules, forage collected and probing behaviour of different foragers were recorded. The flowers are visited by honey bees (*Apis dorsata*, *A. indica* and *A. florea*), butterflies (*Pieris brassicae* and *Danaus plexippus*), flies (*Musca domestica*) and ants (*Camponotus compressus*). They forage daily during 0500–1300 h with peak frequency during 0700–1000 h and of the total visits, honeybees made 42%, butterflies 26%, flies 17% and ants 15%. All the insect species recorded are found foraging throughout the flowering period.

Honey bees and flies forage for both pollen and nectar, while butterflies and ants forage for nectar only. The bees probe the flower in an upright position, by landing on the petals, stamens and stigma. They collect the pollen from the dehisced anthers. In doing so, their ventral portion of the abdomen is coated with pollen and part of it is scraped later by bees' front legs and loaded into their corbiculae. The bees also collect nectar from the flower base. In the subsequent visits, their pollen-laden ventral sides brush against the stigmas and lead to pollination. Flies also use the same posture that the bees employed and carry pollen from one flower to another. Butterflies and ants collect the nectar from nectar chamber by inserting their proboscises. After extracting nectar, they withdraw their proboscides. Then they left the flower and touched the anthers and stigma and ended up being get powdered with the pollen grains when they touched the anthers. Ants are moved within and among the flowers of the same individual, effecting auto- and geitonogamy. On the other hand, honey bees, butterflies and flies facilitate both self- and cross-pollination. Observations of different body parts of the insects under microscope indicate that *A. dorsata* specimens have higher amount of pollen on their body than the other insect specimens and is the most efficient pollinator because of their frequent intra- and inter-plant movements. Bees are also attracted by the yellow colour of the flower. Floral colour is an important attractant prior to flower handling. Bees in general have been reported to be associated with the flower species possessing blue or yellow colour (Kevan 1983, Scogin 1983).

Breeding system : *Cleome viscosa* is a monostylous hermaphrodite species and exhibits weak protandry which appears to have no role in precluding self-pollination. The relative positions of stamens and stigma indicate that there is a possibility for their contact with each other. However, the

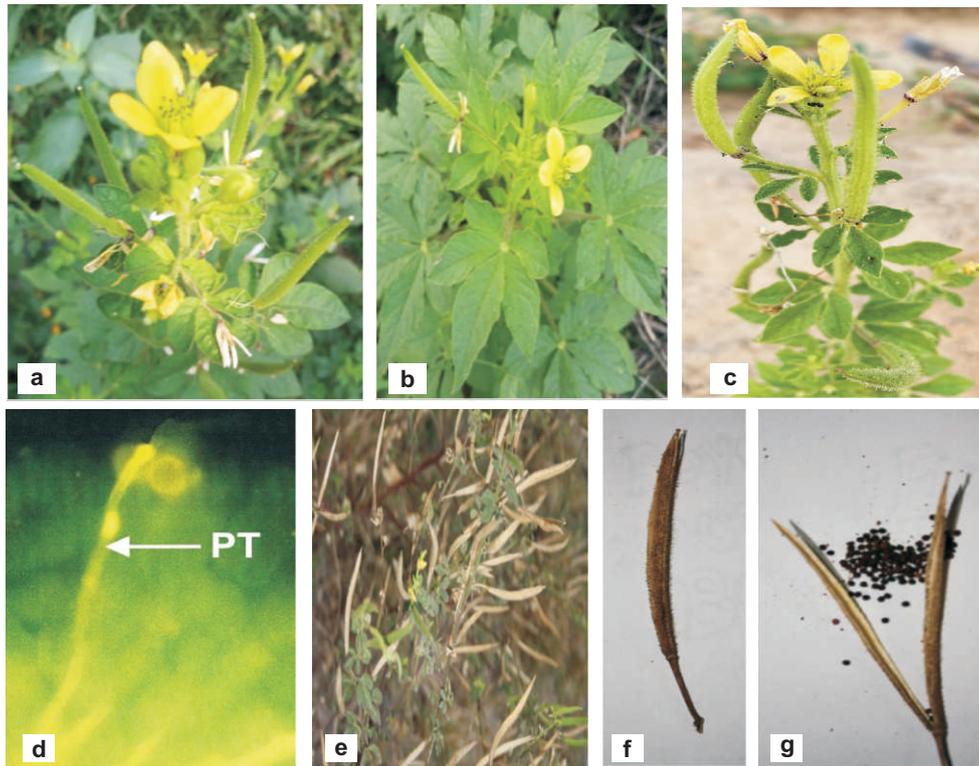


Fig. 1. *Cleome viscosa*. a. Plant with floral buds, open flower and young fruits; b. 5-foliolate leaves, flower and young fruits; c. Young fruits and floral buds; d. *In vivo* pollen germination (PT=pollen tube); e. Plant with mature fruits; f. Single mature fruit covered with trichomes; g. Mature dehisced fruits releasing large number of seeds.

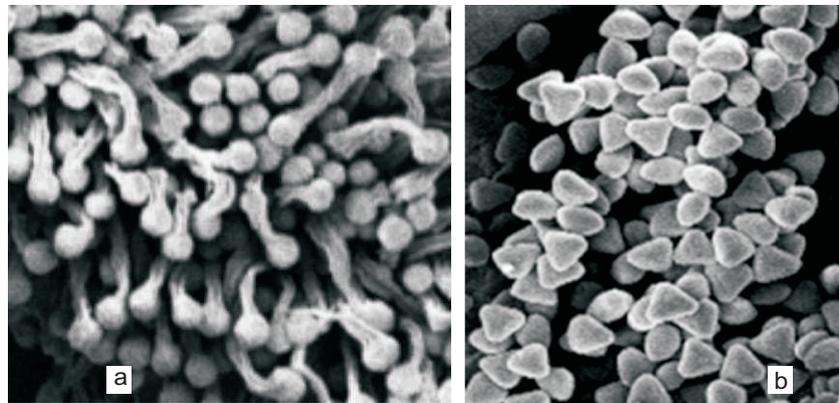


Fig. 2 a.. Scanning electron microphotograph (SEM) of ovarian trichomes. 1 mm = 300 μ m; b. SEM of tricolpate pollen grains. 1 mm = 30 μ m.

flowers bagged without hand pollination for autogamy show a very small percentage of fruit-set. This highlights the necessity for pollen vectors for effective pollen transfer even within flowers to result in self-pollination. Studies made to assert the breeding systems have shown that there is only 6% fruit-set in bagged (unmanipulated autogamy) flowers, 32% fruit-set through manipulated autogamy (hand pollinated, bagged), 80% through geitonogamy and 94% fruit-set through xenogamy. All xenogamous fruits develop to maturity; while 56% of the developing autogamous fruits and 20% of the developing geitonogamous fruits abort subsequently and remaining percentage of fruits develop to maturity. Natural

fruit-set is 77%. A sample of 50 inflorescence consisting of 462 flowers with 11550 ovules selected at random on different plants at flowering stages were used for estimating fruit and seed-set rate. Among these, 354 flowers with 8850 ovules set fruits with 6258 seeds. Seed-set is 70% and in the remaining flowers, some pollinated flowers initially develop fruits but later abort.

The results of hand pollination indicate that *C. viscosa* is facultative xenogamous and its function depends upon pollen vectors. Therefore, both self- and cross-pollination depend on pollen mediation performed by the flower visitors. Although, this species is both self- and cross-pollinated, it sheds most of

the autogamous and geitonogamous fruits, while retaining all xenogamous fruits to maturity. This suggests that *C. viscosa* by predominantly cross-pollinating, selectively eliminating the growing self-pollinated off springs in order to allocate resources for the xenogamous fruits. Cruden (1977) predicted that pollen-ovule ratios are the indicators of breeding system, and estimated the pollen-ovule ratio for each breeding system and the ratio of 244.7-2588 indicate facultative xenogamy. Thus, the pollen-ovule ratio of 1951:1 recorded in present study is suggestive of facultative xenogamy.

Self-compatibility through auto- and geitonogamy is virtually inevitable for *C. viscosa* to produce fruits in situations when pollinators are scarce. Most of the endemic and endangered plant species have been reported to be self-compatible through geitonogamy and this selfing ability is expected to be a 'fail-safe' strategy to produce fruits when pollinators are scarce (Anderson *et al.* 2001, Neel 2002, Torres *et al.* 2003, Rao and Solomon Raju 2003). In *C. viscosa* too, geitonogamy exists; but it is mainly cross-pollinating which indicates that it has facultative xenogamous breeding system.

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