

Floral ecology and autogamy in endemic lesser mallow (*Hibiscus hirtus* L.): Malvaceae

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Abstract

Hibiscus hirtus L., commonly known as 'Lesser Mallow,' is an endemic species gaining popularity in the horticultural and gardening market due to its striking brick-red and white flowers. The plant exhibits seasonal variations in both flower size and seed quantity. It displays a unique stylar movement and practices autogamous pollination. The present research indicates that both open pollination and autogamy yield similar fruit sizes and seed counts, though these characteristics can fluctuate depending on environmental conditions. Experiments have been designed to demonstrate the autogamous pollination strategies in *H. hirtus* by isolating plants from potential pollinators, monitoring stylar movement, and confirming successful fertilization through autogamy. Notably, the flower demonstrates stylar and petal movement during rapid autogamy, with sunlight and moisture levels influencing stylar dynamics. This study provides an in-depth examination of the floral ecology of this potentially ornamental lesser mallow, establishing a valuable baseline for plant breeders and horticultural scientists interested in utilizing this germplasm in floriculture.

Keywords: Allogamy, Insects, Pollination, Stigma, Stylar movement

Introduction

Angiosperms are regarded as the most evolved group of plants due to their adaptations for various pollination and reproduction strategies. While many plant groups exhibit unique pollination methods, as highlighted by Buttrose et al. (1977), Faegri and van der Pijl (1979), and Schlessman (1986), the Malvaceae family shows distinct characteristics in this regard. Most Malvaceae species are self-compatible and can perform delayed self-pollination, featuring flowers with styles that extend beyond the monadelphous stamens (Klips & Snow, 1997; Kumar et al., 2014). As these flowers age, the styles curve outward and backwards until the stigmas contact pollen from the upper anthers. Hamrick and Godt (1990) noted that *Hibiscus tiliaceus* is primarily insect-pollinated. Later, Aluri et al. (2020) reported the presence of specific insect pollinators, including Galapagos carpenter bees and flies, which contribute to dual pollination modes - entomophily (pollination by insects) and anemophily (pollination by wind). The study mentioned various bees that forage on these flowers including honey bees (*Apis dorsata*, *A. cerana*, *A. florea*, and *Trigona iridipennis*), carpenter bees (*Xylocopa latipes* and *Ceratina simillima*), and leaf-cutter bees (*Megachile* sp.). Ruan (2010) classified the flowers of the Malvaceae family into two categories. The first group consists of strict cross-pollinating flowers that remain open for 2-5 days. These flowers display distinct male and female stages. For instance, in *Alcea rosea* L., when the flowers open, the stigma is initially hidden within the staminal column, and only the anthers release pollen. After pollen dispersal, the stigma gradually emerges from the staminal

column, awaiting pollen from a different flower; typically, these flowers are self-incompatible. The second group includes flowers that bloom for only one day, such as *Hibiscus trionum* L. These flowers exhibit both autogamous (self-pollination) and cross-pollination mechanisms. The stigma and anthers mature simultaneously, but the stigma remains upright and away from the anthers when the flowers open, allowing for cross-pollination. If cross-pollination does not occur, the erect stigma bends toward the anthers of the same flower. Once successful pollination occurs, the flowers close, and the petals remain persistent until the early stages of fruit development. Some studies indicate that different species of *Hibiscus* L. exhibit various modes of pollination, including entomophily (insect pollination), anemophily (wind pollination), ornithophily (bird pollination), and self-pollination (autogamy). Buttrose et al. (1977) examined the reversible curvature of style branches in *H. trionum* L. They found that if the stigma does not receive pollen from external sources such as pollinators or wind, the style bends towards the anthers of the same flower to achieve self-pollination. If some pollen from different flowers touches the curving style, the style becomes erect again to avoid self-pollination. The style consistently bends towards the anthers of the same flower without pollinators and wind. In *H. cannabinus*, some cultivars strictly self-pollinate, while others can either self-pollinate or cross-pollinate, depending on the availability of pollinators and environmental conditions. Conversely, *H. sabdariffa* L. predominantly relies on self-pollination (Howard & Howard, 1911; Pai, 1990). Recent studies suggest a 0.20-0.68% chance of cross-pollination

(outcrossing) in *H. sabdariffa* (Sanyal & Datta, 1954; Vaidya, 2000). *Hibiscus hirtus* L., commonly known as 'Lesser Mallow,' is endemic but widely distributed throughout peninsular India, particularly in Western parts of North and Northeast India (Sivarajan & Pradeep, 1996; Paul, 1993). The flowers typically measure 1.5-4 cm in diameter and feature dark brick red, orange, white, and occasionally yellow petals. Due to their attractive appearance, ease of propagation through stem cuttings and seeds, and minimal maintenance requirements, the plant has gained significant horticultural value. Traditionally, it is used for worshipping 'God Ganesha and Goddess Kali,' which is why it is often found near residential areas and temples (Naga Sri et al., 2023). During taxonomic studies, we encountered the plant called 'Dupari,' 'Dupa,' or 'Nareri' in Maharashtra and Goa. This name translates to 'afternoon bloomer,' as the flowers bloom brightly under afternoon sunlight. However, due to the absence of fragrance and limited nectar production, the flowers attract few pollinators. To compensate for this, the flowers exhibit typical stylar movements to facilitate self-pollination. This communication aims to elaborate on the floral biology and autogamy of *H. hirtus*.

Material and methods

Study area

Three study areas were selected based on different light and moisture zones: (1) an open area with low moisture content and direct sunlight, (2) a high moisture content area with direct sunlight, and (3) a nursery area with high moisture content and low sunlight. The first area included Vairag (Solapur) and Vita (Sangli), which have drier climates with relative humidity

below 50%. The second area, Kolhapur, (Maharashtra), has higher moisture content, with relative humidity exceeding 65%. In the third case, plants were kept in a nursery with a regular water supply, while direct sunlight was avoided on the flowers. Observations were conducted throughout the year, from January 2021 to December 2022 and from January 2023 to December 2023, to assess flower size and opening span at different moisture and light levels. For the bagging of flowers, either the branch with a solitary flower was covered with transparent cloth, or the entire pot was covered if it contained a single flower. This measure was taken to prevent contact between the pollinators and the flower. Control plants were established in all areas without bagging to monitor the number of pollinators and pollen carried to the flowers. In contrast to the study of autogamy, plants with solitary flowers were placed in a net box to restrict access to pollinators while allowing proper sunlight without disturbing the flowers. The bagging of capsules was conducted early to ensure the collection of all seeds. Photography was utilized to capture the stages of stilar movement and autogamy. Images were taken every half hour from blooming to the closing of the flower using a Nikon 3000D and a Vivo 21e camera. This procedure was repeated three times each season to ensure accuracy. The analysis recorded the response of the plant and flower in moist conditions, including measurements of diameter, blooming period, and flower size. The stages of autogamy were also documented. Self-pollination was monitored for fruit and seed number observations, and a comparative account of floral biology and autogamy of the endemic lesser mallow was elaborated.

Results and discussion

Floral biology

Light is a crucial factor for flowering initiation in plants, as Mauseth (2003) and Capon (2005) highlighted. Based on their light requirements, plants can be classified into three main categories: Short-Day Plants (SDP), Long-Day Plants (LDP), and Day-Neutral Plants (DNP). Research on *Hibiscus* species also reveals that a critical light period is necessary to initiate flowering. Warner & Erwin (2001, 2003) conducted experiments on the effects of light on the flowering of *Hibiscus* L. They identified *H. radiatus* Cav. as a short-day plant, while *H. cisplatinus* A.St.-Hil. and *H. trionum* L. are classified as day-neutral plants. *H. moscheutos* L. is recognized as a long-day plant. Pai (1990) noted that *H. cannabinus* L. and *H. sabdariffa* are short-day plants that require less than 12 hours of light for flowering. Compared with these findings, we observed that *H. hirtus* blooms throughout the year, regardless of light intensity. However, the flower number, size, and blooming period vary. During the rainy season, the size of the flower's ranges from 0.9 to 3 cm. The lack of pollinators during this period and rapid vegetative growth may contribute to the smaller flowers, which feature a short staminal column and fewer ovules in the ovary. Consequently, these smaller flowers tend to produce smaller fruits, with an

average seed count of less than seven (Tables 1, 2). The plant thrives in a dry climate with bright sunlight, with the best flowering period from October to April (Table 1). The relationship between flower size, blooming duration (in hours), and insect visits is illustrated in Figure 1. Highest number of flowers was recorded in plants exposed to bright sunlight in areas with less than 50% relative humidity, yielding 1,969 flowers per year for ten plants of the same age. In contrast, in more humid areas (above 65% relative humidity), the flower count declined to 1,090. Additionally, in nurseries and polyhouses, where direct sunlight is limited, and relative humidity exceeds 80%, only 933 flowers were recorded (Table 1).

Pollination

H. hirtus typically begins to bloom at sunrise, reaching full bloom in the afternoon when it is bathed in bright sunlight. We observed three types of insects interacting with the flowers: the Grass Blue and Red Pierrot butterflies, Honeybees and Bumblebees. While bees are often recorded during morning sessions, butterflies tend to visit in the afternoon. The average seed count remains unchanged in both open pollination systems (where the flowers are not bagged) and autogamy (where the flowers are bagged to prevent cross-pollination). This

Table 1. Flower size at different environmental condition (Average values from 2021-2023)

Treatment	At moist habitat/ Short day condition	Inside Shade house/ nursery	Dry climate in bright sunlight
June to Sept	0.9 - 1.8 cm	0.8 - 1.7 cm	1.0 - 2.5 cm
Oct to Jan	1.2 - 2.3 cm	1.0 - 2.0 cm	1.8 - 3.0 cm
Feb to May	1.2 - 2.3 cm	1.0 - 2.0 cm	1.2 - 2.8 cm

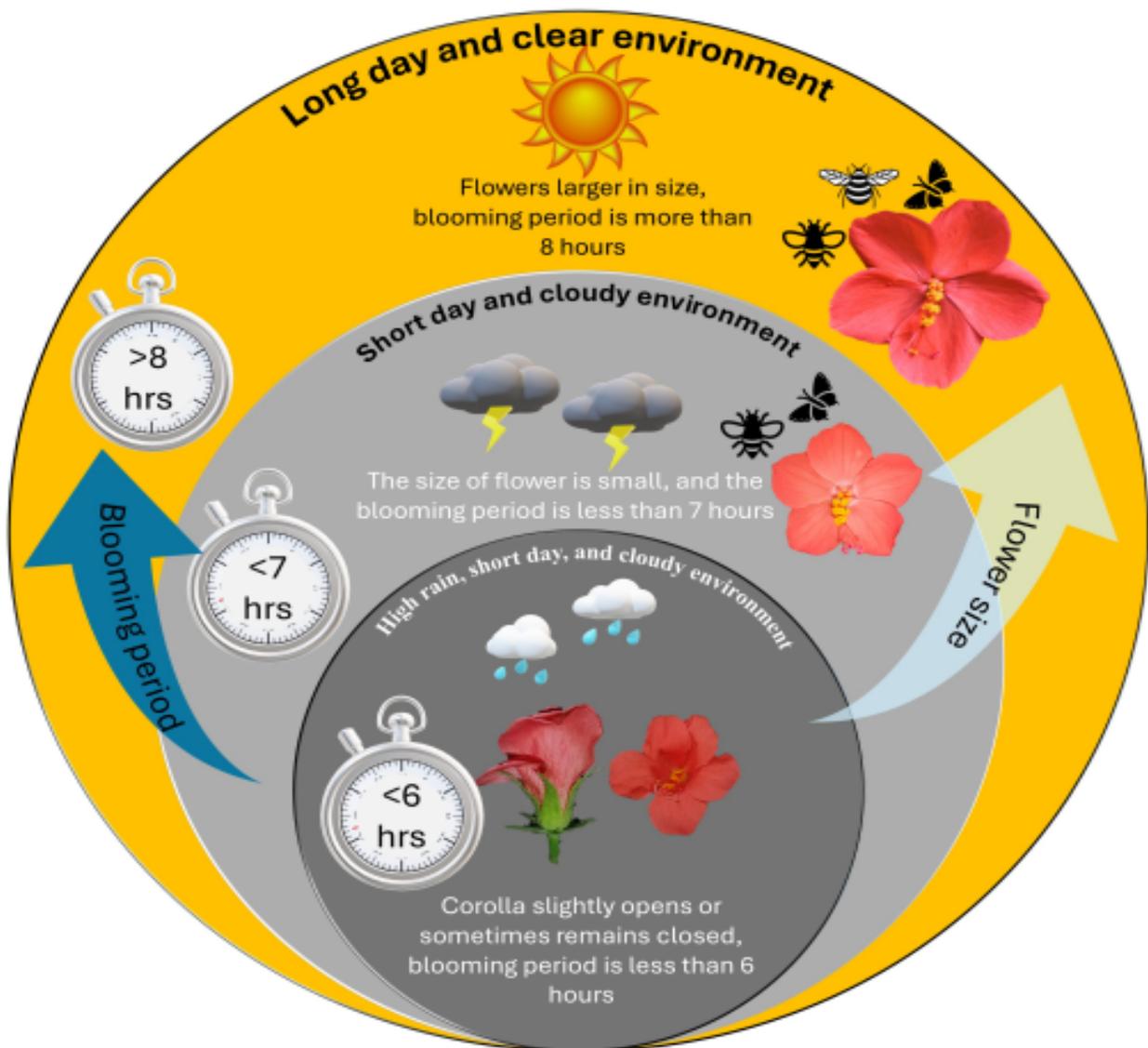


Figure 1. Model of pollination ecology of *H. hirtus* showing variation in flower size, insect visitors and blooming span during different environmental conditions

indicates that *H. hirtus* flowers are capable of effective self-pollination (autogamous) without the aid of pollinators, producing seeds at a rate comparable to that of cross-pollination.

Autogamy and stylar movement

Autogamy is a standard mode of pollination in *H. hirtus*. In isolated conditions, 9 out of 10 plants exhibited autogamy. The style extends from the

ovary through the staminal column to the stigma. Its length ranges from 0.7 to 2 cm, depending on the size of the flower. The entire style is enclosed within the staminal column, except at the apex, which divides into five arms. Each arm measures 2-5 mm from the staminal column's apex to the stigma's base. The stylar arms are typically glabrous and straight when the flowers open, pointing outward from the corolla to expose the stigmas, allowing



Figure 2. Stages of autogamy and stylar movement in *Hibiscus hirtus* L.
 A - A bloomed flower in bright sunlight with a straight exerted style at 8.30 A.M; B - After failing to receive pollen from another flower, the stigma slowly starts to reflex in centrifugal directions; C, D, E - Style become perpendicular to staminal column and corolla become flat; F, G, H - Complete recurved stigma towards the staminal column of the same flower; I, J - Persistent petals up to early fruit stages

Table 2. Autogamy in different environmental conditions (Average values from 2021-2023)

Month	At moist habitat/ Short day condition			Inside Shade house/ nursery			Dry climate in bright sunlight		
	Flowers	Fruits	Seeds	Flowers	Fruits	Seeds	Flowers	Fruits	Seeds
January	120	90	450	91	65	301	200	170	2034
February	102	88	459	95	67	402	197	167	2036
March	100	76	424	80	59	397	180	150	1989
April	76	49	309	69	50	298	123	100	1404
May	50	30	200	50	37	200	100	80	1087
June	60	47	311	67	57	423	120	90	1376
July	80	57	478	70	58	457	135	99	1414
August	90	60	500	76	51	431	150	129	1597
September	98	62	513	80	54	444	176	150	1656
October	99	65	520	79	55	423	189	179	2111
November	97	70	565	90	66	307	201	187	2287
December	118	87	590	86	61	312	198	185	2370
Total	1090	781	5319	933	680	4395	1969	1686	21361
Fruiting %		71.65			72.88			85.62	
Avg. seed/ fruit		6-7			6-7			12-13	

them to receive pollen from other flowers. The stigmatic lobes are capitate, measuring 0.4 to 0.8 mm in diameter and are covered with papillate hairs (Fig. 2 A). If the stigma does not receive enough pollen from another plant, it begins to reflect in a 'U' shape (Fig. 2 B-G). This process occurs relatively slowly, taking 7-8 hours for the stigma to receive pollen from the anthers of the same flower. This bending strategy allows the flower to capture enough pollen from its anthers. The corolla closes completely once the stigma receives enough pollen (Fig. 2 H, I). The closed corolla protects the style and staminal column from the wind, pollinators, and rain. The petals remain persistent for up to 2-5 days, and when the fruits emerge from the calyx, the dried corolla and staminal column are shed (Fig. 2 I, J). The speed of stylar movement for

autogamy varies depending on environmental conditions, such as light and rainfall. During continuous rainy seasons, flowers generally remain closed but still manage to produce fruit through autogamy. In contrast, when there is cloudy weather with sparse rainfall, flowers open for a short period (approximately 3-6 hours) leading to a rapid movement of the style. The relationship between the speed of stylar movement and autogamy across different seasons is illustrated in (Fig. 3). Klip & Snow (1997) conducted experiments on *H. laevis* and determined that the species is self-compatible, achieving a 100% fruit set through artificial self-pollination. However, they found that natural autogamy resulted in a 64% fruit set. They also noted that the movement of the style is delayed if the stigma receives pollen

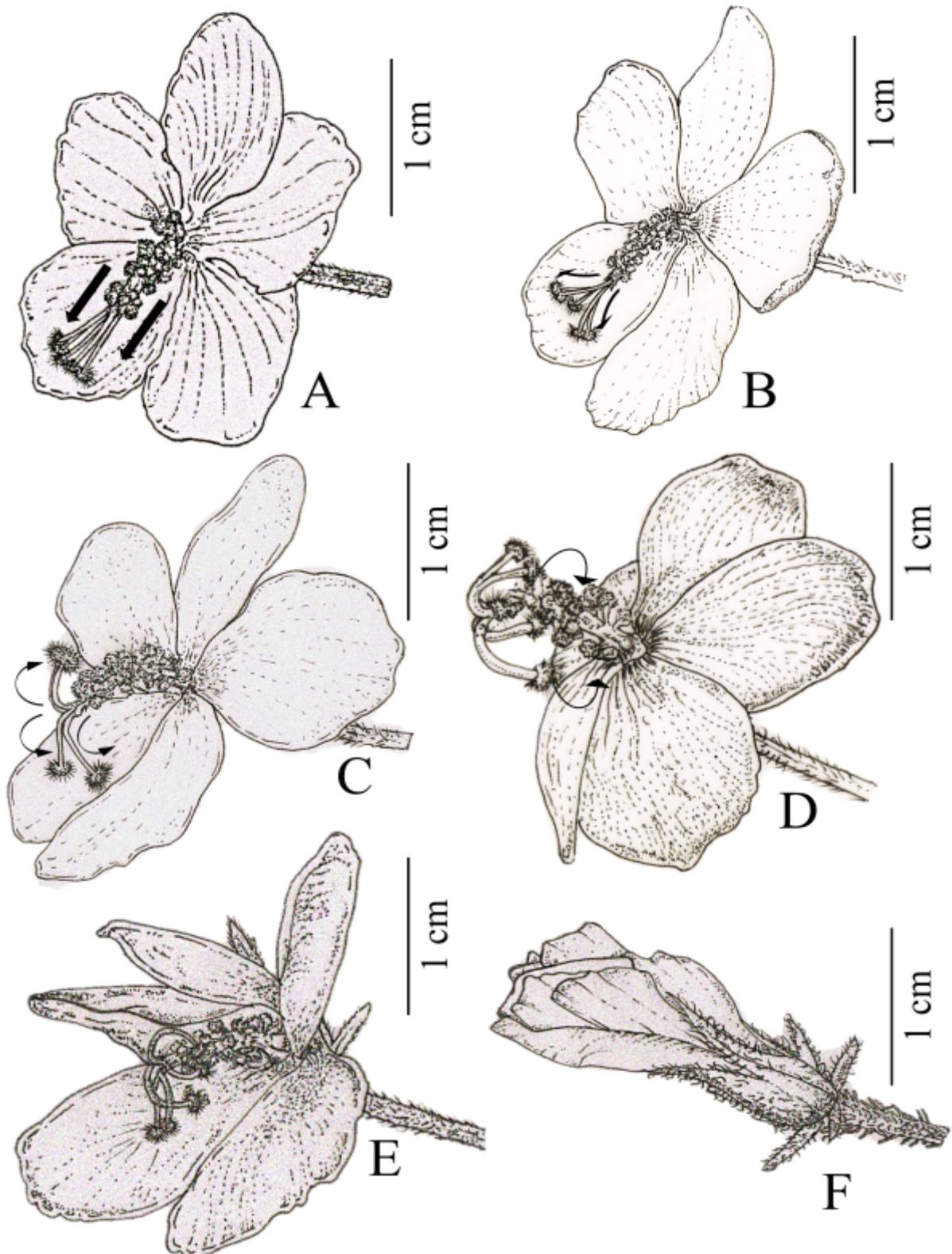


Figure 3. A, B, C, D, E, F - Direction of stylar movement of *H. hirtus* L.

from pollinators or other vectors.

Fruiting

Both autogamous and xenogamous pollination in *H. hirtus* can yield healthy fruits. However, the number of seeds produced can vary based on factors such as flower size, the number of ovules in the ovary, and the fertilization percentage. In moist environments with high relative humidity (greater than 80%), particularly during rainy and overcast conditions, flower size tends to be smaller. This reduction in size affects the number of ovules in the ovary and the stamens on the staminal column, leading to the formation of smaller capsules that contain an average of 6-7 seeds each. Additionally, heavy rainfall can hinder stigma bending, resulting in insufficient pollen uptake. Continuous and intense rainfall can wash away pollen entirely, preventing flowers from producing fruit; the lowest fruiting percentage recorded in these moist areas was 71.65% (Table 2). Conversely, open areas with relative humidity below 50% and sunny weather promote the development of larger flowers with more ovules in the ovary and stamens on the staminal column.

Autogamy is prevalent in *Hibiscus hirtus* L., with nine out of ten flowers successfully undergoing this process under various conditions. Due to its lower nectar production and lack of fragrance, *H. hirtus* experiences fewer pollinator visits compared to other *Hibiscus* species. Experimental and comparative studies (Vaidya, 2000) suggest that autogamy represents the most effective pollination strategy for *H. hirtus*, similar to that of the roselle plant (*H. sabdariffa*). Klips & Snow (1997) investigated the timing of cross-pollination, observing that the style in *H. laevis* often recurs to another flower

of the same plant, resulting in a fruiting rate of 64%. This finding aligns with the present study, which reports fruiting percentages ranging from 71.65% to 85.62%.

Conclusions

The lesser mallow is a day-neutral plant, blooming continuously throughout the year; however, the sizes of its flowers, fruits, and seed counts can vary depending on environmental conditions. A drier, sunnier climate tends to favour the development of larger flowers and fruits. The lesser mallow is considered horticulturally significant thanks to its ease of germination, autogamy, and ability to propagate vegetatively. In the coming years, a diverse array of *H. hirtus* varieties is expected to be developed through mutational breeding. This study will enhance our understanding of the pollination strategies, ecology, and floral biology of similar *Hibiscus* species and their conservation and application in gardening and horticulture.

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