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RESEARCH ARTICLE

The Study of Floral Biology in Wild and Cultivated Species of Cotton (*Gossypium* spp.)

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Abstract

A set of 14 different species and three interspecific hybrids of cotton were studied during 2019-2021 to evaluate floral morphology and reproductive biology. The mean performance of genotypes revealed variation for different characters among all the species studied. The highest number of anthers was found in ISH-P1 (interspecific hybrid plant) (174), followed by *Gossypium raimondii* (173). However, when tested for pollen viability, all three interspecific hybrids turned out to be sterile. Conversely, the wild species *G. nelsonii* showed the highest pollen viability (95.29%). The highest *in-vitro* pollen germination percentage was exhibited by *G. herbaceum* (89.4%), which also had the longest pollen germ tube length (924.03 µm). The length of the pedicle varied from 3.64 cm in *G. barbadense* to 0.34 cm in *G. nelsonii*. The tree species *G. raimondii* exhibited the longest style (43.96 mm), which was also a poor performer in terms of selfing (38.89%) and outcrossing potential (0%). Tetraploid *G. barbadense* had the largest pollen grains (121.12 µm), whereas the smallest were observed in *G. trilobum* (80.43 µm). The highest values of selfing (95.45%) and outcrossing potential (10%) were observed in desi cotton species *G. arboreum* and *G. herbaceum*, respectively.

Keywords: Floral biology, *Gossypium*, Pollen viability, Wild cotton.

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Introduction

Cotton is one of the major fiber crops of the world with a high commercial value. It is commercially grown in 70 countries, particularly in temperate and tropical regions. China, the USA and India are the three major cotton-growing countries where climatic conditions meet the natural growth requirements of cotton. These conditions include periods of hot and dry weather and adequate moisture obtained through irrigation. Primary centers of diversity are distributed in the tropical and subtropical regions of the world, where 18 species are found in west-central and southern Mexico, 14 species in northeast Africa and 17 species in Arabia and Australia. Two species, *viz. Gossypium hirsutum* and *G. barbadense* contribute to more than 90% of the total cultivated area. Two other species, *G. arboreum* and *G. herbaceum*, are popularly referred to as desi cotton in India which are indigenous to Asia and Africa, respectively. The tetraploid (2n = 52) species of cotton, *viz. G. hirsutum L.* and *G. barbadense L.* were introduced into India during the 17th and 18th centuries A.D. (Hutchinson, 1959).

Floral biology is the study of flower life, which starts with the ripening of one or more reproductive organs, such as the dehiscence of the first stamen or the attainment of receptivity by stigma, and ends when the stamens have shed all their pollen and the stigmas have stopped being receptive (Percival, 1979).

It discusses the evolutionary and functional significance of floral traits and provides a detailed overview of the structure, behavioral pattern and functions of floral parts. The color, size, shape and arrangement of various floral whorls vary greatly in flowers and play an important role in attracting pollinators (Leppik, 1956). Additionally, the removal of attractive secondary structures may decline the probability of fruit set (Bell, 1985). Hossain *et al.* (2016) found that higher outcrossing was a function of percent stigma exertion, stigma length and breadth. In contrast, Kudo (2003) reported that long anthers have higher pollen removal rates than short anthers. The spatial arrangement of anthers plays an important role in the placement of pollen on the pollinator's body, which in turn affects the efficiency of pollination (Waser and Price, 1984; Wolfe and Barrnett, 1989). Therefore, the study of the floral biology of a crop helps in choosing an appropriate breeding program (Rai *et al.,* 2007), because crop improvement, especially the development of hybrids, largely depends on the floral biology of a crop, involving numerous operations around the flower. Hence, an efficient pollination control mechanism is necessary for the development and seed production (Kempe and Gils, 2011) of any crop, necessitating the study of the floral biology of a crop.

Materials and Methods

The research material consists of diverse species of *Gossypium*, including 10 wild, four cultivated species and three interspecific hybrids (Table 1) and (Figure 1). The investigation aimed to gather information on variations in floral biology. The observations were recorded for 13 different characters from three randomly selected plants from both cultivated and wild species and a single plant from each interspecific hybrid.

The study on floral morphology and reproductive biology was conducted, examining different characteristics such as the number of petals, sepals and anthers, length of style and pedicel, pollen viability, *in-vitro* pollen germination, length of the germ tube, pollen size, selfing potential and outcrossing potential. Qualitative traits of flowers, including flower color, pollen shape and color, were also recorded. Observations of floral morphology, including the number of petals, sepals and anthers, the length of style and pedicel, were recorded from five samples per plant from three randomly selected plants.

Pollen viability was assessed using 1% acetocarmine (Heslop-Harrison, 1992). A droplet of 1% acetocarmine was placed on a microscopic slide and freshly dehisced anthers were dusted onto the staining solution. The slide was then observed under the microscope. Four randomly chosen microscopic fields were examined and pollen grains that were deeply stained. were counted as viable. Pollen grains that appeared unstained, yellowish, or shriveled were considered non-viable (Bi *et al.*, 1998; Yankova-Tsvetkova *et al.*, 2013).

For *in-vitro* pollen germination, the medium proposed by Song *et al.* (2014) was used, which consisted of different components such as 0.07% MnSO4, 0.04% Ca (NO3)2, 0.02% H3BO3, 0.01% serine, 0.01% glutamate, 0.01% lysine, 0.01% proline and 40% sucrose dissolved in 100 mL of deionized water. Pollens were sprinkled on the culture media and

Table 1: List of species used in the study

S. No.	*Plant material*	*Source*
1.	*Gossypium robinsonii* F. Muell	
2.	*Gossypium thurberi* Tod.	
3.	*Gossypium capitis-viridis* Mauer	
4.	*Gossypium stocksii* Mast.	
5.	*Gossypium trilobum* (DC.) Skovst.	Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra.
6.	*Gossypium davidsonii* Kellogg	
7.	*Gossypium triphyllum* (Harv.) Hochr.	
8.	*Gossypium nelsonii* Fryxell	
9.	*Gossypium tomentosum* Nutt.	
10.	*Gossypium raimondii* Ulbr.	Department of Genetics and Plant Breeding, AAU, Anand.
11.	*Gossypium hirsutum* L. (G COT 20)	Main Cotton Research Station, NAU, Surat.
12.	*Gossypium herbaceum* L. (1027 ALF)	
13.	*Gossypium barbadense* L. (GSB 44)	Regional Cotton Research Station, AAU, Viramgam.
14.	*Gossypium arboreum* L. (Phule Anmol)	Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra.
15.	*Gossypium raimondii* Ulbr. × *Gossypium barbadense* L. (ISH-P1) *	
16.	*Gossypium raimondii* Ulbr. × *Gossypium barbadense* L. (ISH-P2)	Department of Agricultural Biotechnology, AAU, Anand.
17.	*Gossypium raimondii* Ulbr. × *Gossypium barbadense* L. (ISH-P3)	

***ISH-P:** Interspecific hybrid plant

Figure 1: Different species of cotton. (Length of plastic pole = 2 m)
(A). *G. davidsonii* (B). *G. trilobum* (C). *G. stocksii* (D). *G. triphyllum* (E). *G. raimondii* (F). *G. brasiliense* (G). *G. robinsonii* (H). G. thurberi (I). *G. nelsonii*

incubated for 3 to 4 hours at 28°C. Germination was calculated on a percentage basis, while germ tube length and pollen size were recorded from 10 randomly selected germinated pollens.

Selfing potential was determined by placing bags over 30 selected flower buds and for outcrossing potential, approximately 50 flower buds were emasculated per species and the boll set was recorded.

Results and Discussion

Number of Sepals and Petals

There was no variation among the studied species, as all the species under study possessed five sepals and petals.

Number of Anthers per Flower

The number of anthers plays a major role in the pollination process of any plant species. A higher number of anthers

produces more pollen, leading to effective pollination and, consequently, higher fruit or seed set. Significant variation was observed in the number of anthers per flower across different species. The highest number of anthers was found in ISH-1, an interspecific hybrid between *G. raimondii* × *G. barbadense* (174), followed by *G. raimondii* (173), while the lowest number was found in *G. stocksii*, a wild cotton species (67). Among cultivated species, *G. hirsutum* (96), *G. barbadense* (120), *G. herbaceum* (91) and *G. arboreum* (96) showed no significant variation, except for *G. barbadense*, which exhibited the highest number of anthers among cultivated species (Table 2). Mehetre (1982) reported the number of anthers in *G. hirsutum* to be (88 ± 9) and in *G. barbadense* (86 ± 7). Kakani *et al.* (2003), while studying the effects of UV-B radiation on the number of anthers per flower, recorded 100 anthers in *G. hirsutum*. Meyer (1965) investigated cytoplasmic effects on anther numbers in interspecific hybrids using the cytoplasm of *G. harkenssii*, *G. hirsutum*, *G. herbaceum*, *G. arboreum* and *G. anomolum* and reported a range of 56 to 142 anthers per flower in different interspecific hybrids. Mehetre *et al.* (2005) studied the variability in cultivated and wild species and reported the number of anthers in different species: *G. herbaceum* (121), *G. thurberi* (102), *G. davidsonii* (46), *G. raimondii* (154), *G. trilobum* (98), *G. stocksii (50)* and different races of *G. hirsutum* showed a range of 77 to 112 anthers, while *G. arboreum* races recorded 71 to 112 anthers. Kaur *et al.* (2016) reported 122 anthers in *G. hirsutum* and 124 in *G. armourianum*. However, the F_1 hybrid between *G. hirsutum* and *G. armourianum* showed 117 anthers per flower. Tahir and Noor (2011) performed a similar study and recorded 58 anthers in *G. hirsutum*, 65 in *G. arboreum* and 75 anthers in the interspecific hybrid.

Length of the Pedicel (cm)

The length of the pedicel is an important character because a long pedicel confers a non-preference type of resistance for bollworms by making the movement of the larvae difficult (Mehetre *et al.,* 2005). *G. barbadense* (3.64 cm) exhibited the longest pedicel length, followed by *G. hirsutum* (3.25 cm). *G. nelsonii* exhibited the shortest pedicel length, approximately 0.34 cm, followed by *G. stocksii* (0.38 cm). Geddam (2010) studied pedicel length and reported a range of 0.7 to 1.90 cm in *G. arboreum* and *G. herbaceum* (Table 2). Gapparov (2019) studied different inter and intraspecific hybrids and recorded a pedicle length of 0.5 to 0.6 cm in crosses between *G. arboreum* subsp. perenne and *G. arboreum* subsp. *obtusifolium* var. *indicum*. Meanwhile, Imtiyazahmed *et al*. (2020) reported a pedicle length of 2.8 cm in *G. hirsutum*, 1.53 cm in *G. armourianum* and 3.4 cm in the triploid interspecific hybrid.

Flower Color

Visual observation was conducted using the RHS color chart at the peak flowering stage in all the species. Out of 14 species, seven exhibited cream-colored flowers. Three were yellow, two were deep yellow and two were purple. All three interspecific hybrids showed cream-colored flowers (Table 2). Pooja *et al.* (2016) characterized 50 genotypes of *G. hirsutum* and reported a higher frequency of cream-colored flowers, followed by yellow and pink flowers in the studied genotypes. Mehetre *et al.* (2005) studied the variability in cultivated and wild species and reported petal color in different species *viz. G. herbaceum* (yellow), *G. thurberi* (cream), *G. davidsonii* (pale yellow), *G. raimondii* (cream), *G. trilobum* (cream), *G. stocksii* (light yellow) and different races of *G. hirsutum* showed the range of cream - yellow petals while *G. arboreum* races recorded yellow – deep yellow petals.

Pollen Color and Shape

Pollens were observed under a microscope from freshly dehisced anthers from each treatment. Out of 14 species, 7 exhibited cream-colored pollen, 1 showed yellow pollen and six were deep yellow, whereas all the interspecific hybrids recorded cream-colored pollen. In terms of shape, pollens of all the species and interspecific hybrids exhibited a spherical shape (Table 2). Rathinavel (2017) studied pollen color in 101 extant cotton varieties and parental lines of hybrids and reported that cream color (76.24%) was the most frequent, followed by yellow (20.79%) and deep yellow (2.97%).

Length of Style (mm)

The style length plays an important role in pollination. Shorter styles are very effective for successful pollination in the absence of pollinators (Pleasants and Wendel, 2010). *G. raimondii* (43.96 mm) exhibited the longest style length, followed by an interspecific hybrid *G. raimondii* × *G. barbadense* (ISH-P2) (43.30 mm) and *G. raimondii* × *G. barbadense* (ISH-P1) (33.03 mm). *G. thurberi* (13.26 mm) exhibited the shortest style length, followed by *G. stocksii* (13.76 mm). The cultivated species have an average style length of 19.31 mm (Table 2). Khadi *et al.* (1998) studied style length in cotton and reported a range from 15.7 to 18.7 mm in different genotypes. Kakani *et al.* (2003) reported a style length of 20.7 mm in the control plant and 21.2 mm in the UV-B-treated plant of *G. hirsutum*. Mehetre *et al.* (2005) studied the variability in cultivated and wild species and reported style length in different species as follows: *G. herbaceum* (1.9 cm), *G. thurberi* (2.1 cm), *G. davidsonii* (1.9 cm), *G. raimondii* (4.5 cm), *G. trilobum* (2.1 cm), *G. stocksii* (1.3 cm). Different races of *G. hirsutum* showed style length ranging from 2.1 to 2.8 cm, while *G. arboreum* races recorded 1.9 to 2.4 cm. Tahir and Noor (2011) recorded style length in *G. hirsutum* (1.5 cm), *G. arboreum* (1.4 cm) and 1.8 cm in the interspecific hybrid. Imtiyazahmed *et al.* (2020) reported a style length of 2.7 cm in *G. hirsutum*, 3.42 cm in

Table 2: Mean performance of different species of cotton for various characteristics of floral morphology and reproductive biology

Sr. No	Names of species	Petal color	Pollen color	No. Anthers	Len. of stigma (mm)	Len. of pedicel (cm)	PS (µm)	PV (%)	PG (%)	GTL (µm)	SP (%)	OCP (%)
1	*G. robinsonii*	Purple	Deep yellow	99	18.45	1.70	93.00	83.33	57.14	232.10	58.82	1.13
2	*G. thurberi*	Cream	Cream	91	13.26	0.97	90.40	61.95	59.04	111.04	75.00	5.71
3	*G. capitis-viridis*	Cream	Cream	101	18.42	0.71	86.53	78.49	49.55	100.60	55.00	1.69
4	*G. stocksii*	Cream	Cream	67	13.76	0.38	94.75	60.00	57.23	239.84	65.38	3.89
5	*G. trilobum*	Cream	Cream	88	14.01	1.64	80.43	67.42	75.55	211.58	54.17	0.00
6	*G. raimondii*	Cream	Cream	173	43.96	1.29	87.49	60.20	64.10	153.86	38.89	0.00
7	*G. triphyllum*	Cream	Cream	79	14.30	0.72	82.43	60.34	70.47	341.76	75.00	4.16
8	*G. nelsonii*	Purple	Cream	111	18.94	0.34	88.64	95.29	87.20	123.57	90.00	0.00
9	*G. tomentosum*	Deep yellow	Deep yellow	98	22.32	2.96	105.08	94.02	62.14	223.54	73.68	0.00
10	*G. davidsonii*	Yellow	Yellow	96	15.56	1.76	92.26	77.92	85.20	716.65	92.31	0.00
11	*G. hirsutum* (G COT 20)	Cream	Deep yellow	96	19.35	3.25	99.02	83.46	81.00	623.99	80.95	9.20
12	*G. herbaceum* (1027 ALF)	Yellow	Deep yellow	91	15.34	2.90	92.14	93.85	89.40	924.03	86.36	10.00
13	*G. barbadense* (GSB 44)	Deep yellow	Deep yellow	120	25.99	3.64	121.12	94.85	88.80	482.65	86.36	8.60
14	*G. arboreum* (Phule Anmol)	Yellow	Deep yellow	96	16.56	2.85	95.46	84.78	76.30	729.83	95.45	9.50
15	*G. raimondii* × *G. barbadense* (ISH –P1)	Cream	Cream	174	33.03	1.46	84.45	0.00	0.00	0.00	0.00	0.00
16	*G. raimondii* × *G. barbadense* (ISH –P2)	Cream	Cream	160	43.30	1.48	82.91	0.00	0.00	0.00	0.00	0.00
17	*G. raimondii* × *G. barbadense* (ISH –P3)	Cream	Cream	159	23.84	1.52	81.34	0.00	0.00	0.00	0.00	0.00
	General mean			114.8	22.72	1.80	91.61	78.34	71.65	373.50	73.38	3.84
	Range			67–175	13.26–43.96	0.34–3.64	80.43–121.12	0–95.29	0–89.4	0–924.03	0–95.45	0–10
	SE			8.32	2.53	0.27	2.44	3.39	3.27	66.04	4.08	0.97
	CV (%)			29.91	46.59	59.03	11.01	17.86	18.85	73.10	22.96	104.59

PS: Pollen size, PV: Pollen viability, PG: Pollen germination, GTL: Germ tube length, SP: Selfing potential, OCP: Out crossing potential

G. armourianum and 3.27 cm in the triploid interspecific hybrid.

Pollen viability

The aceto-carmine test was used to assess pollen viability, which plays a crucial role in any crop as viable pollen is necessary for successful fertilization. In cotton, which is often cross-pollinated and primarily pollinated by insects, especially bees, successful pollination relies on insect activity, as cotton pollen, being heavy, cannot be carried by the wind (Santhy *et al.,* 2008). Increased viable pollen numbers correspond to higher chances of achieving good fruit or seed set. Wide variation was observed in pollen viability among different species of cotton. *G. nelsonii* exhibited the highest pollen viability (95.29%), followed by *G. barbadense* (94.85%) and *G. tomentosum* (94.02%). However, three interspecific hybrids showed zero percent pollen viability. Cultivated species exhibited more than 80% pollen viability, while wild species showed pollen viability between 60 to 80%, except for two wild species, which showed more than 90% pollen viability (Table 2 and Figure 3). Marutani *et al.* (1998) used acetocarmine to assess pollen viability in inter- and intra-specific hybrids of *Anthurium andraeanum* and reported that intra-specific hybrids were relatively more fertile than inter-specific hybrids. Douglas (1968) studied pollen viability in *G. hirsutum* monosomics and reported a range from 3 to 34%. Sangole and Tidke (2020) used IKI solution to determine pollen viability in different cotton species and found that variety H-8 (99.3%) exhibited the highest pollen viability, while Renuka-143 (93.2%) recorded the lowest pollen viability. Kaur *et al.* (2016) and Imtiyazahmed *et al.* (2020) reported 2.19 and 2.33% pollen viability in interspecific hybrids, respectively.

In-vitro Pollen Germination

For the germination of pollen, different media were tried. The medium proposed by Song *et al*., (2014) was used and wide variation was observed among different species for *in-vitro* germination of pollens (Figure 4 C). It ranged from 0 to 89.4 %. The highest germination per cent was exhibited by *G. herbaceum* (89.4 %) followed by *G. barbadense* (88.8%) and *G. nelsonii* (87.2%) (Table 2 and Figure 2). The three interspecific hybrids had shriveled and damaged pollens, which failed to grow in culture media and all interspecific hybrids showed no germination, indicating the sign of sterility. Song *et al.* (2014) studied pollen germination in *G. hirsutum* and recorded pollen germination percentages ranging from 32.68 to 85.02%. They observed that an increase in incubation temperature affects the germination percentage drastically. When the incubation temperature was below 30°C germination percentage between cultivars differed. They suggest that pollen germination at 35°C as compared to at 30 could be used as the screening parameter for high-temperature tolerance. Taylor (1972) conducted a study on the pollen germination of *G. hirsutum* using an artificial medium composed of 3.5% agar, sucrose, $MnSO_4$, $Ca(NO_3)_2$, and H_3BO_3. He reported an average germination rate of 34.4% for the Acala 1517D cultivar and 29.8% for Stoneville 7A. Sangole and Tidke (2020) also studied pollen germination on an agar medium and reported that variety H-8 (27.8%) exhibited the highest pollen germination, while variety H-10 (5%) recorded the lowest pollen germination. Barrow (1981) performed an elaborate combination of experiments on *in-vitro* pollen germination. Astonishingly, within 3 to 4 minutes, 98% of pollens ejected pollen tubes; however, the author also noted that after pollen tube ejection, pollens were no longer viable (Figure 4A). Barrow (1981) also suggested that the optimum temperature range for pollen germination is between 32 and 40°C. Burke *et al.* (2004) studied multiple factors affecting *in-vitro* pollen germination in cotton and concluded that high germination and pollen tube elongation occurred at 28°C. Maintaining relative humidity between 55 to 80% optimizes pollen tube elongation (Figure 4B). Burke *et al.* (2004) additionally observed that the rapid ejection of structures resembling pollen tubes in Barrow's (1981) experiment was not indicative of actual pollen tubes but rather the cytoplasm of pollen (Figure 4D).

Germ Tube Length (µm)

Several microscopic fields were observed to record the length of the germ tube (µm) after the incubation period. Germ tube lengths varied significantly among different cotton species, with cultivated species and *G. davidsonii* performing notably well. The longest pollen germ tube was exhibited by *G. herbaceum* (924.03 µm), followed by *G. arboreum* (729.83 µm), *G. hirsutum* (623.99 µm) and *G. davidsonii* (716.6 µm). Conversely, *G. capitis-viridis* (100.6 µm), *G. thurberi* (111.04 µm) and *G. nelsonii* (123.57 µm) displayed the shortest germ tube lengths (Table 2). Pollen germination and germ tube length primarily depend on the type of media used and incubation conditions. However, other factors such as flowering period, temperature, disease

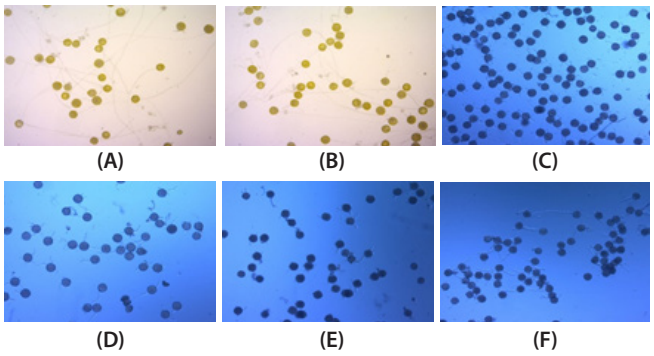
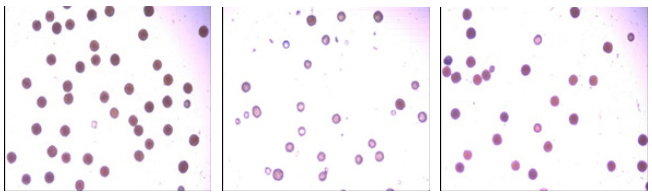


Figure 2: Pollen germination in different species of cotton (A). *G. herbaceum* (B). *G. davidsonii* (C). *G. nelsonii* (D). *G. raimondii* (E). *G. thurberi* (F). *G. stocksii*



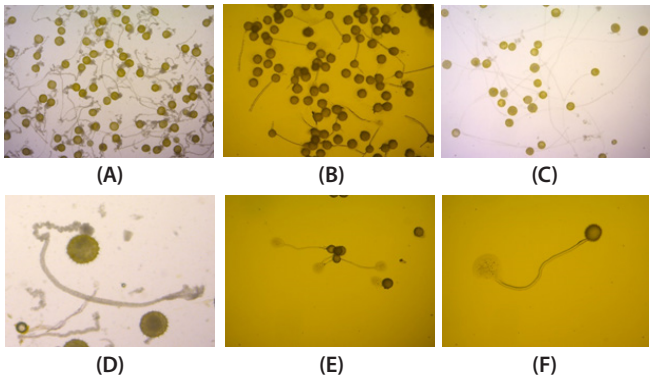
(A) (B) (C)

Figure 3: Pollen viability in different species of cotton
(A). *G. herbaceum* (B). *G. raimondii* X *G. barbadense* (C). *G. capitis viridis*

and insect infestation also exert significant influence (Song *et al.,* 2014). Kakani *et al.* (2005) investigated *in-vitro* pollen germination and tube growth in 12 cotton cultivars, reported germ tube lengths ranging from 605 to 905 µm across different cultivars, with an average of 778 µm. Taylor (1972) noted that germ tube length was typically 15 to 30 times the diameter of the pollen in their study of *in-vitro* pollen germination. Wauford (1979) modified Taylor's medium and recorded a pollen tube length of 2.8 mm *in-vitro*. Burke *et al.* (2004) suggested that >80% relative humidity aids in rapid pollen tube elongation, while maintaining a relative humidity of 55 to 80% optimizes stabilized elongation. However, higher relative humidity levels (>80%) were found to cause pollen tube rupture after rapid elongation (Figure 4 E & F).

Pollen Size (µm)

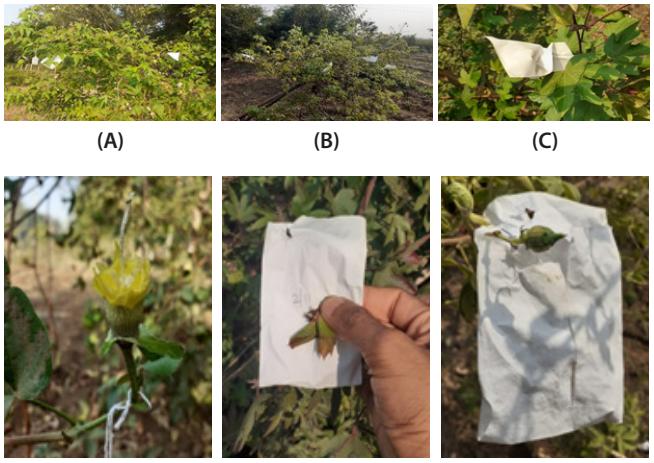
Pollen grains from each species were mounted in acetocarmine stain and photographed under a microscope. The diameter of pollen was measured using Axion Vision software, with ten pollen grains studied for each species. Apart from a few tetraploid species, minimal variation in pollen size was observed. The average pollen size for all species was 91.5 µm, with most species clustering around this mean. The largest pollen size was observed in *G. barbadense* (121.12 µm), followed by *G. tomentosum* (105.08 µm) and *G. hirsutum* (99.02 µm). Conversely, the smallest pollen size was found in *G. trilobum* (80.43



(A) (B) (C)

(D) (E) (F)

Figure 4: Pollen germination in different growth media.
(A). Barrow (1981) (B). Burke *et al*., (2004) (C). Song *et al*., (2014) (D). Ejection of cytoplasm from pollen tube in Barrow's medium (E). & (F). Bursting of tubes under (>80%) high humid incubation



(A) (B) (C)

(D) (E) (F)

Figure 5: Study of selfing and outcrossing potential.
A, B & C showing bagging of unopened flowers for the study of selfing potential., D. Showing emasculated flower for the study of outcrossing potential., E. Showing failure of boll set., F. Showing boll setting.

µm), followed by *G. raimondii* × *G. barbadense* (ISH-P3) (81.34 µm) and *G. triphyllum* (82.43 µm) (Table 2). These findings align with those of Jones and McCurry (2012), who studied pollen size in cultivated species and reported a mean pollen size of 106.7 µm for *G. barbadense*, with the largest measuring 125 µm. For *G. hirsutum*, the mean pollen size was 94.6 µm, with the largest pollen measuring 109 µm. Additionally, they reported mean pollen sizes of 80.4 µm for *G. herbaceum* and 84.8 µm for *G. arboreum*. Savaskan (2002) investigated the effects of gamma irradiation on pollen size in *G. hirsutum*, reported pollen sizes of 120.35 µm in the control group. Kaur *et al.* (2016) recorded pollen sizes of 116 µm for *G. hirsutum*, 103 µm for *G. armourianum* and 69 µm for the interspecific hybrid. Naggar (2004), in a study on pollen morphology of Egyptian Malvaceae family crops, reported pollen sizes of 68 µm for *G. hirsutum* and 88 µm for *G. barbadense*. Vaissiere and Vinson (1994) explored the effects of pollen morphology on pollen collection by insects, noting that *G. hirsutum* pollen measured 94.6 µm. They also inferred that the length of the spines on cotton pollen affects cotton pollen collection by honey bees.

Selfing Potential

Significant variation was observed among different cotton species regarding their selfing. The highest selfing potential was recorded in *G. arboreum* (95.45%), followed by *G. nelsonii* (90%) and other cultivated species, all showing selfing potential above 80%. In contrast, all interspecific hybrids exhibited the lowest selfing potential as they were sterile. Apart from interspecific hybrids, *G. raimondii* (38.89%) and *G. trilobum* (54.17%) demonstrated the lowest selfing potential. This could be attributed to factors such as longer style length in *G. raimondii*, which protrudes beyond the anthers, making pollen deposition on the stigmatic surface

difficult (Table 2 and Figure 5). Additionally, the absence of pollinators may hinder both cross and self-pollination in these species. The average selfing potential across all studied species, excluding sterile interspecific hybrids, was 69.84%. These findings are consistent with the research of Wilson and Stapp (1985), who studied pollination in cotton and reported self-pollination rates ranging from 80.5 to 88.1%.

Outcrossing Potential

Outcrossing potential was determined by emasculating flowers and allowing for natural outcrossing. Among the studied species, *G. herbaceum* exhibited the highest outcrossing potential at 10%, followed by *G. arboreum* (9.5%) and *G. hirsutum* (9.2%). Most of the wild species displayed outcrossing potential ranging from 0 to 5%. Specifically, only *G. thurberi* among the wild cotton species exhibited 5% outcrossing, while the remaining species demonstrated less than 5%, with five species exhibiting zero outcrossing (Table 2 and Figure 5). Research by Bozbek *et al.* (2008) delved into outcrossing in cotton and reported a range of 0 to 13.3% outcrossing in *G. hirsutum*. Stephens and Finkner (1953) studied natural cross-pollination in cotton and suggested significant variability in natural crossing rates across different regions. They proposed that natural crossing could be lower than 5% in some areas of Texas but exceed 50% in North Carolina, with bee populations playing a crucial role in this disparity.

Conclusion

The wild and cultivated species had a wide variation in almost all the characters under the study. The variation between different species is evident in the CV% recorded in the current study. Pollen and stigma characters are very important in breeding programs as their interaction with one another plays a major role in selecting parents for distant hybridization. Studies on pollen germination, germ tube length and stigma receptivity can be used while planning distant hybridization in cotton. It is also apparent that the characters that indirectly contribute to higher yield, *viz.* selfing potential, outcrossing potential, pollen germination and germ tube length, are significantly morphed in cultivated species by the anthropogenic intervention when compared to wild species.

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