International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating: 5.29
IJABR 2025; 9(6): 456-462
www.biochemjournal.com
Received: 01-03-2025
Accepted: 05-04-2025

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Study of floral biology in different varieties of Gladiolus in the foothills of Himalaya

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i6f.4568

Abstract
The present study was conducted during 2023-2024 at the Model Floriculture Centre and the College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, to elucidate the floral biology of nine *Gladiolus* varieties to inform breeding strategies, particularly hybridization programs. Significant genotypic variations were observed across key reproductive parameters. The duration of anthesis ranged from 3.56 h in Nathan Red to 8.18 h in Acidanthera. Anther dehiscence commenced post-anthesis, with durations spanning from 2.48 h in Bindiya to 8.23 h in Acidanthera. Stigma receptivity initiated after anther dehiscence, persisting for 43.70 h in Careless and extending up to 60.45 h in Vicky Liar. Morphometric analyses of reproductive structures revealed significant differences among the cultivars. Vicky Liar exhibited the longest androecium (5.38 cm), whereas Careless had the shortest (3.49 cm). Filament lengths varied from 3.03 cm in Careless to 4.67 cm in Bindiya. Anther length ranged between 0.82 cm in Purple Majesty and 1.66 cm in Acidanthera. Gynoecium lengths were shortest in Purple Majesty (7.09 cm) and longest in Acidanthera (15.93 cm). Style lengths spanned from 4.57 cm in Careless to 7.66 cm in White Prosperity. Stigma lobe lengths varied, with Bindiya at 0.54 cm and Careless at 1.83 cm. Pollen viability exceeded 50% across all varieties, peaking at 67.15% in Acidanthera and reaching a minimum of 58.62% in Careless. Pollen size measurements indicated the largest grains in White Prosperity and Punjab Morning and the smallest in Bindiya and Regency. These findings underscore the genetic diversity among the studied *Gladiolus* cultivars, highlighting the necessity of comprehensive floral biology assessments prior to initiating hybridization programs. Such evaluations are critical for selecting optimal parental combinations and timing pollination to enhance seed set success.

Keywords: Anthesis, dehiscence, receptivity, androecium, gynoecium, pollen, etc.

Introduction
Gladiolus (*Gladiolus grandiflorus* L.), commonly known as the sword lily, is a prominent bulbous flowering plant in the Iridaceae family. The name gladiolus is derived from the Latin word 'gladius', meaning sword for the shape of its leaves (Lewis *et al.*, 1972) [7]. Renowned for its striking, colourful blooms and sword-like leaves, it is widely cultivated for both garden displays and as cut flowers. The genus Gladiolus encompasses approximately 250-300 species, primarily native to South Africa, the Mediterranean and parts of Asia. Gladiolus plants grow from corms and produce tall spikes, or inflorescences, that can reach heights of 2 to 6 feet (60 to 180 cm). The flowers bloom sequentially from the bottom to the top of the spike. Gladiolus grows rapidly in its natural habitat and the ploidy level varies from dodecaploid (2n = 12x = 180) in the European regions to diploid (2n = 30) in the Cape region. Currently, 20-25 species have been used to create farmed gladiolus hybrids (Misra *et al.*, 2003) [10].

India has made significant strides in gladiolus breeding over the past few decades, focusing on developing cultivars with enhanced traits such as disease resistance, vibrant colors and longer spike lengths. Notable cultivars like Arka Kesar, H.S.84-7-11 and Shobha have been identified for their early flowering and desirable floral characteristics (Poon, 2009) [16]. Breeding programs have also aimed to improve resistance to Fusarium wilt, a significant disease affecting gladiolus. Efforts include evaluating genotypes for resistance and incorporating disease-resistant traits into new cultivars, vibrant colours, fragrance and more number of florets with longer spike length (Taj *et al.*, 2016) [21].

Thus, work to meet the consumer demand in the field of floriculture is always prioritized.

A thorough understanding of the plant's floral biology is necessary to overcome the breeding challenges and enhance the efficiency of hybridization efforts. The species is predominantly protandrous, wherein anther dehiscence precedes stigma receptivity, thereby promoting cross-pollination. Anthesis typically occurs in the early morning hours, with anthers dehiscing between 8:00 and 9:30 AM. Stigma receptivity spans approximately 24 to 48 hours post-anthesis, peaking between 24 to 36 hours. (Messar *et al.*, 2016) [9]. These temporal dynamics necessitate precise synchronization and detailed studies of floral biology to optimize breeding strategies during controlled hybridization to maximize seed set and hybrid viability.

Materials and Methods

The study was carried out at the Model Floriculture Center and College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), during 2023-24. The field experiments were laid out in a Randomized Block Design, at a spacing of 30 cm × 20 cm and the laboratory experiments were laid out in a Completely Randomized Design (CRD) with 3 replications each. Nine gladiolus varieties were selected for the study of floral biology *viz*., Punjab Morning, Careless, Bindiya, White Prosperity, Purple Majesty, Regency, Nathan Red, Acidanthera and Vicky Liar. The corms were planted in October 2023 and general practices for crop production were followed throughout the experiment to grow a healthy crop.

The selected florets were tagged to assess key reproductive parameters *viz*., anthesis duration, anther dehiscence and stigma receptivity. To monitor anthesis, observations commenced at the initial sign of perianth opening and continued until full expansion was achieved. The time interval between these two stages was recorded to determine the duration of anthesis. This approach aligns with standard practices in floral biology studies, where precise timing of flower opening is important for understanding reproductive phases. Anther dehiscence was assessed by observing the anthers from the onset of longitudinal splitting at the apex to the completion of pollen release. The duration was recorded by noting the time at which dehiscence began and the time when it concluded. This method provides insights into the synchrony between pollen availability and stigma receptivity, which is vital for successful pollination. To evaluate stigma receptivity, controlled pollination experiments were conducted at various intervals: on the day of anthesis and subsequently on the first, second, third and fourth days post-anthesis. Each pollinated floret was monitored for seed set, which served as an indicator of stigma receptivity at the time of pollination. This technique is commonly used in reproductive biology to determine the functional period of the stigma.

Five representative plants were randomly selected from the field. At the full bloom stage, the basal floret from each plant's spike was carefully harvested for analysis. Each floret was meticulously dissected to isolate the primary floral organs. Measurements were taken for the following six parameters:

1. Length of the androecium
2. Length of filaments
3. Length of anthers
4. Length of the gynoecium
5. Length of the style
6. Length of stigma lobes

Measurements were recorded in centimeters using a precision ruler. For each parameter, the mean value was calculated across the sampled florets to ensure accuracy and reliability.

The acetocarmine staining technique was employed to assess pollen viability, following established cytological protocols. Freshly dehisced pollen grains were collected from mature anthers and immediately transferred onto clean microscope slides. A drop of 1% acetocarmine solution prepared by dissolving 1 g of carmine powder in 100 mL of 45% glacial acetic acid was added to the pollen sample. The preparation was gently mixed and covered with a coverslip. The slides were then left undisturbed for 2-3 minutes to facilitate uniform staining. Microscopic examination was conducted under bright-field illumination. Viable pollen grains were identified by their round morphology and intense crimson-red staining, indicative of intact nuclei and cytoplasmic content. In contrast, non-viable or aborted pollen grains appeared shriveled and lacked significant staining. For each sample pollen grains were counted across multiple microscopic fields to ensure statistical reliability. The percentage of viable pollen was calculated using the formula:

$$\text{Pollen viability \%} = \frac{\text{Number of viable pollens}}{\text{Total number of pollens}} \times 100$$

The estimation of pollen diameter was done through an ocular micrometer and stage micrometer. Before measuring the pollen diameter, calibration was used for the ocular micrometer. It was put on the eyepiece of the compound microscope while the stage micrometer was put on stage. The stage micrometer was 1 mm long (1000 µm) and had 100 divisions/units while the ocular micrometer had 10 units/divisions. Calibration can be done at 10x or 40x magnification of eyepiece. Here, calibration was done at 40x and observations were recorded.

Subsequent data from pollen viability and diameter assessments were analyzed statistically using a Completely Randomized Design (CRD).

Results and Discussion

Data presented in Table 1 indicate distinct varietal differences in the timing and duration of anthesis in *Gladiolus*. In all varieties studied, the anthesis process, comprising both initiation and completion, was concluded within a single day. Anthesis initiation was recorded between 08:06 am and 11:12 am, while completion occurred between 02:02 pm and 04:24 pm. Among the genotypes, 'Acidanthera' exhibited the longest anthesis duration, taking 491 minutes (8.18 hours), followed by 'Regency' (440 minutes or 7.33 hours) and 'Punjab Morning' (309.66 minutes or 5.16 hours). The shortest duration was observed in 'Nathan Red', with anthesis completed in 213.66 minutes (3.56 hours). The peak anthesis period was recorded between 10:07 am and 03:22 pm, lasting approximately 5.22 hours. These findings are consistent with previous reports. Shah *et al.* (1988) [19] and Ohri and Khoshoo (1981) [13] documented that anthesis in *Gladiolus* generally begins around 08:15 am and continues until approximately 10:15

am, with peak activity between 08:00 am and 10:00 am. Similarly, Poon *et al.* (2009) [16] noted floret opening occurring between 05:53 am and 11:56 am, while Messar *et al.* (2016) [9] also reported same-day anthesis initiation from 08:00 am onwards. The observed variation in anthesis duration and timing is largely attributable to genotypic differences among cultivars, reflecting inherent physiological and genetic diversity.

The process of anther dehiscence, encompassing both initiation and completion, was observed to occur on the same day across all *Gladiolus* genotypes studied. Notably, dehiscence commenced only after anthesis had begun. The initiation of anther dehiscence was recorded between 08:39 am and 12:55 pm, while completion occurred between 01:40 pm and 04:53 pm. Among the evaluated varieties, 'Acidanthera' exhibited the longest duration for anther dehiscence, taking 494 minutes (8.23 hours) followed by 'Regency' (368.67 minutes or 6.14 hours) and 'White Prosperity' (276.67 minutes or 4.61 hours). In contrast, the shortest duration was recorded in 'Bindiya' (149.33 minutes or 2.48 hours). Based on these findings, the optimal time window for pollen collection was identified between 10:39 am and 03:05 pm, lasting approximately 4.42 hours (Table 2). These results align closely with earlier studies by Ohri and Khoshoo (1981) [13] and Mahawar and Misra (1993a), who also reported that anthesis and anther dehiscence occur in close succession, typically coinciding with floral opening. Shah *et al.* (1988) [19] recorded anther dehiscence in *Gladiolus* between 08:30 am and 12:00 pm, while Poon *et al.* (2009) [16] observed a broader time span, from 05:59 am to 02:17 pm. Similarly, Messar *et al.* (2016) [9] documented genotypic variation in anther dehiscence timing among *Gladiolus* cultivars. These variations in the timing and duration of anther dehiscence can be attributed primarily to genotypic differences, underscoring the influence of inherent genetic factors on floral phenology.

Stigma receptivity in *Gladiolus* was assessed over four consecutive days on the day of anthesis and the second, third and fourth days post-anthesis. The initiation of stigma receptivity was recorded between 09:15 am and 12:23 pm, while its termination occurred between 08:05 am and 04:52 pm, depending on the variety. Among the genotypes studied, the cultivar 'Vicky Liar' exhibited the longest stigma receptivity duration, lasting 3627.00 minutes (60.45 hours), followed by 'Bindiya' (3306.33 minutes or 55.10 hours) and 'Punjab Morning' (3167.00 minutes or 52.78 hours). In contrast, the shortest receptivity duration was observed in 'Careless' (2622.00 minutes or 43.70 hours). Based on the collective data (Table 3), the most suitable period for pollination was determined to be between 10:33 am and 12:33 pm. These findings are in agreement with previous studies. Ohri and Khoshoo (1981) [13] reported that in *Gladiolus*, the stigma becomes receptive approximately one and a half hours after anther dehiscence and remains receptive for 24 to 30 hours. Similarly, Shah *et al.* (1988) [19] noted that stigma receptivity may commence one day before anthesis and continue up to two days afterward. Conversely, Singh and Singh (1985) [20] reported a longer window of stigma receptivity lasting up to 72 hours post-anthesis. Messar *et al.* (2016) [9] also observed that stigma receptivity generally initiates after anthesis. The duration and onset of stigma receptivity are strongly influenced by ambient temperature, with warmer temperatures accelerating and cooler temperatures delaying, the onset and progression of receptivity.

Morphological evaluation of the floral reproductive organs revealed significant varietal differences in both male and female components, as presented in Tables 4 and 5. With respect to the androecium, the maximum total length was recorded in 'Vicky Liar' (5.38 cm), followed closely by 'Bindiya' (5.31 cm) and 'Nathan Red' (4.93 cm). The minimum length was observed in 'Careless' (3.49 cm). For filament length, 'Bindiya' showed the highest measurement (4.67 cm), followed by 'Vicky Liar' (3.66 cm) and 'White Prosperity' (3.61 cm) and with the lowest value again observed in 'Careless' (3.03 cm). The length of the anther was greatest in 'Acidanthera' (1.66 cm), followed by 'Nathan Red' (1.42 cm) and 'Regency' (1.36 cm), while the shortest anthers were found in 'Purple Majesty' (0.82 cm). Analysis of female reproductive parts showed considerable variation among the genotypes as well. The longest gynoecium was recorded in 'Acidanthera' (15.93 cm), followed by 'Vicky Liar' (9.47 cm) and 'White Prosperity' (9.36 cm), whereas 'Purple Majesty' showed the shortest gynoecium (7.09 cm). 'Acidanthera' also had the longest style (15.10 cm), followed by 'White Prosperity' (7.66 cm) and 'Vicky Liar' (7.36 cm). The shortest style was recorded in 'Careless' (4.577 cm). In contrast, the maximum stigma lobe length was observed in 'Careless' (1.832 cm), followed by 'Nathan Red' (1.32 cm) and 'Vicky Liar' (1.213 cm), while the minimum was observed in 'Bindiya' (0.543 cm). These results corroborate earlier findings by Roy (2003) [18], Farhat and Roy (2005) [5], and Akansha *et al.* (2014), who reported considerable morphological variability in reproductive structures among different *Gladiolus* genotypes.

The analysis of pollen viability (Table 6) revealed no statistically significant differences among the evaluated *Gladiolus* varieties. However, numerical variation was evident. The highest pollen viability was recorded in 'Acidanthera' (67.15%), followed by 'Punjab Morning' (66.86%) and 'Bindiya' (66.24%). The lowest viability was observed in 'Careless' (58.62%). Despite the variability, all cultivars exhibited pollen viability above 50%, suggesting overall good fertility potential, likely influenced by genotypic attributes. These findings are in alignment with the results of Poon *et al.* (2010) [10], who observed the lowest percentage of sterile pollen in the genotype 'Sapna' (2.82%), followed by 'Poonam' (4.00%), Hybrid Selection 88-10-22 (4.82%), and Hybrid Selection 82-11-27 (5.22%). Similarly, Messar *et al.* (2016) [9] reported high pollen viability ranging from 56.69% in 'Red Beauty' to 64.95% in 'Nathan White'. The pollen size also showed varied differences among these varieties. It was observed that White Prosperity had the maximum pollen width *i.e.*, 98.10 µm followed by Punjab Morning (95.96 µm) and Nathan Red (91.58 µm). The minimum pollen width was observed in Purple Majesty (75.84 µm) as shown in Table 6. While the maximum length was found in Punjab Morning (102.64 µm) followed by White Prosperity (101.86 µm) and Bindiya (98.99 µm). The minimum was observed in Regency (83.98 µm). Variation in pollen size may have implications for fertilization success and could serve as a morphological indicator for genotype differentiation.

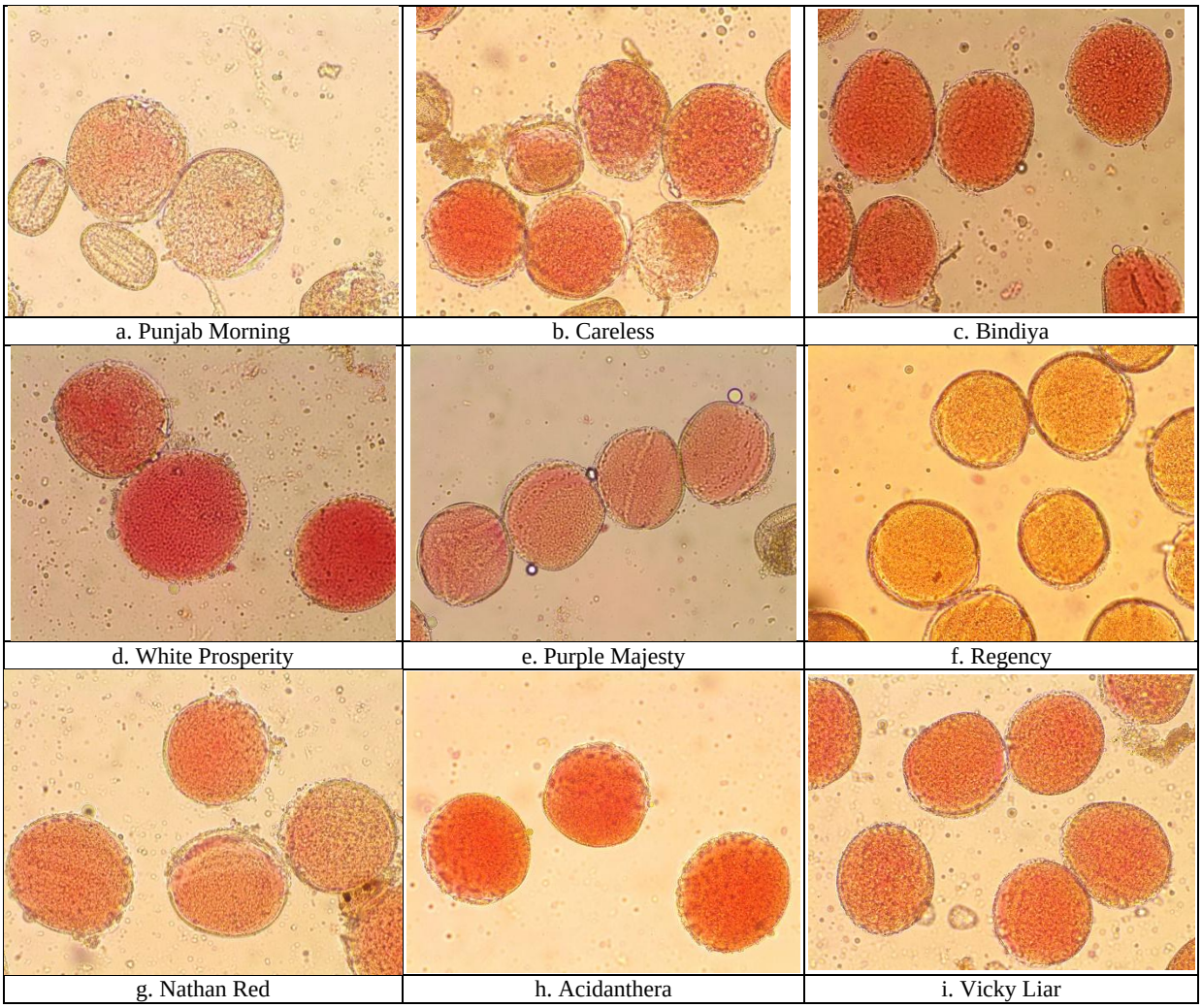
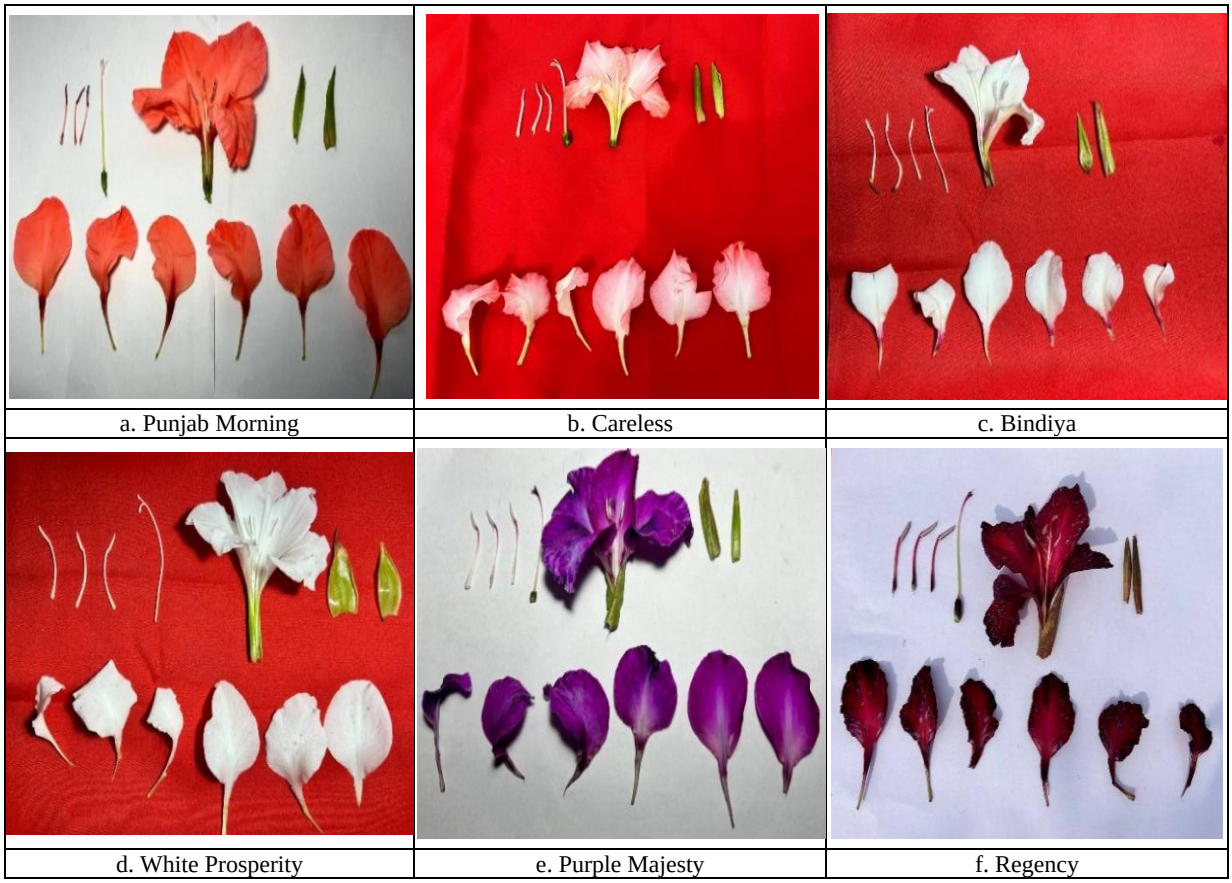


Fig 1: Pollen studies in different varieties of gladiolus



g. Nathan Red | h. Acidanthera | i. Vicky Liar

Fig 2: Reproductive parts of gladiolus in different varieties

Table 1: Duration of anthesis in different varieties of gladiolus

S. No.	Varieties	Initiation	Completion	Duration of anthesis (minutes)
1	Punjab Morning	10:07 am	02:56 pm	309.66 (5.16 h)
2	Careless	09:12 am	02:02 pm	299.33 (4.98 h)
3	Bindiya	08:06 am	04:21 pm	273.66 (4.56 h)
4	White Prosperity	09:17 am	02:17 pm	300.33 (5.00 h)
5	Purple Majesty	11:11 am	03:32 pm	261.33 (4.35 h)
6	Regency	09:01 am	04:21 pm	440 (7.33 h)
7	Nathan Red	11:12 am	02:46 pm	213.66 (3.56 h)
8	Acidanthera	08:13 am	04:24 pm	491 (8.18 h)
9	Vicky Liar	10:43 am	02:33 pm	230.33 (3.83 h)
Mean		10:07 am	03:22 pm	313.25 (5.22 h)
C.D.				30.841
SE(m)				10.199

Table 2: Duration of dehiscence in different varieties of gladiolus

S. No.	Varieties	Initiation	Completion	Duration of dehiscence (minutes)
1	Punjab Morning	10:31 am	02:46 pm	255.33 (4.25 h)
2	Careless	10:17 am	01:40 pm	203 (3.38 h)
3	Bindiya	12:55 pm	03:24 pm	149.33 (2.48 h)
4	White Prosperity	09:57 am	02:34 pm	276.66 (4.61 h)
5	Purple Majesty	11:56 am	03:02 pm	186 (3.10 h)
6	Regency	09:07 am	03:16 pm	368.66 (6.14 h)
7	Nathan Red	11:24 am	03:08 pm	224 (3.73 h)
8	Acidanthera	08:39 am	04:53 pm	494 (8.23 h)
9	Vicky Liar	11:06 am	02:58 pm	232.33 (3.87 h)
Mean		10:39 am	03:05 pm	265.48 (4.42 h)
C.D.				26.97
SE(m)				8.919

Table 3: Duration of stigma receptivity in different varieties of gladiolus

S. No.	Varieties	Initiation	Completion	Duration of stigma receptivity (minutes)
1	Punjab Morning	11:32 AM	04:52 PM	3167.00 (52.78 h)
2	Careless	12:23 PM	08:05 AM	2622.00 (43.70 h)
3	Bindiya	09:37 AM	04:43 PM	3306.33 (55.10 h)
4	White Prosperity	09:24 AM	12:05 PM	3040.67 (50.67 h)
5	Purple Majesty	11:14 AM	01:59 PM	3045.33 (50.75 h)
6	Regency	09:15 AM	10:59 AM	2983.67 (49.72 h)
7	Nathan Red	11:00 AM	11:35 AM	2915.00 (48.58 h)
8	Acidanthera	10:16 AM	09:38 AM	2841.67 (47.36 h)
9	Vicky Liar	10:16 AM	11:36 AM	3627.00 (60.45 h)
Mean		10:33 AM	12:23 PM	3060.96 (51.016 h)
C.D.				376.357
SE(m)				124.464

Table 4: Androecium length, filament length and anther length in different varieties of gladiolus

S. No.	Varieties	Androecium length (cm)	Filament length (cm)	Anther length (cm)
1	Punjab Morning	4.60	3.56	1.21
2	Careless	3.49	3.03	1.00
3	Bindiya	5.31	4.67	0.87
4	White Prosperity	4.86	3.61	1.25
5	Purple Majesty	4.69	3.51	0.82
6	Regency	4.57	3.18	1.36
7	Nathan Red	4.93	3.39	1.42
8	Acidanthera	4.28	3.35	1.66
9	Vicky Liar	5.38	3.66	1.51
C.D.		0.488	0.258	0.133
SE(m)		0.161	0.085	0.044

Table 5: Gynoecium length, style length and stigma lobe length in different varieties of gladiolus

S. No.	Varieties	Gynoecium length (cm)	Style length (cm)	Stigma lobe length (cm)
1	Punjab Morning	8.83	6.52	0.97
2	Careless	7.65	4.57	1.83
3	Bindiya	7.40	5.75	0.54
4	White Prosperity	9.36	7.66	0.71
5	Purple Majesty	7.09	5.61	0.68
6	Regency	7.60	5.97	1.03
7	Nathan Red	8.53	6.62	1.32
8	Acidanthera	15.93	15.10	0.98
9	Vicky Liar	9.47	7.36	1.21
C.D.		0.396	0.402	0.133
SE(m)		0.131	0.133	0.044

Table 6: Pollen viability and diameter in different varieties of gladiolus

S. No.	Varieties	Pollen viability (%)	Pollen width (µm)	Pollen length (µm)
1	Punjab Morning	66.86	95.96	102.64
2	Careless	58.62	87.01	90.55
3	Bindiya	66.24	89.51	98.99
4	White Prosperity	62.01	98.10	101.86
5	Purple Majesty	63.86	75.84	84.36
6	Regency	61.36	81.19	83.98
7	Nathan Red	63.20	91.58	97.94
8	Acidanthera	67.15	83.34	88.66
9	Vicky Liar	61.71	79.83	89.43
C.D.		N/A	10.083	11.313
SE(m)		1.93	3.367	3.778

Conclusion

The observed variability in the timing of anthesis, anther dehiscence and stigma receptivity among *Gladiolus* genotypes underscores the influence of inherent genetic differences. Such temporal distinctions are critical for optimizing breeding operations, including pollen collection, floret bagging and manual pollination. The characterization of reproductive structures namely the lengths of the androecium, filament, anther, gynoecium, style and stigma lobes provides valuable insight into floral morphology and reveals structural constraints that hinder effective pollination. Additionally, significant variation in pollen viability and diameter across cultivars indicates differing levels of fertilization potential and may serve as predictive indicators for hybridization success. Collectively, these findings emphasize the importance of detailed floral biology assessments prior to the implementation of conventional breeding programs. Understanding these parameters allows for the selection of optimal parental lines and the precise timing of hybridization activities, thereby enhancing the likelihood of successful seed set and hybrid development.

Acknowledgments

We would like to express our gratitude to all the co-authors for their invaluable contributions to this research. The collaborative efforts and support of each co-author greatly enhanced the quality and depth of this work. This paper is a result of our shared commitment to enhancing the knowledge in this field.

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