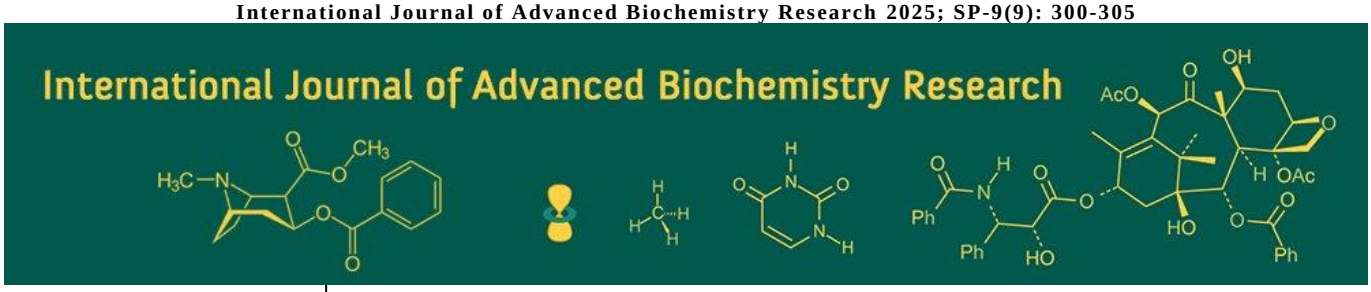

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(9): 300-305
www.biochemjournal.com
Received: 21-07-2025
Accepted: 25-08-2025

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Floral and reproductive biology of *Clitoria ternatea* L. (Shankupushpi)

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i9Sd.5509

Abstract
Clitoria ternatea L., commonly known as butterfly pea, is an underutilized leguminous plant with considerable medicinal, ornamental and nutraceutical value. Despite its economic potential, limited information exists on its reproductive biology, which is critical for breeding and seed production. The present study was conducted to investigate floral biology, pollen viability, *in vitro* pollen germination and stigma receptivity in 12 contrasting genotypes. Significant morphological divergence was observed between single and double petalled types, the former exhibiting papilionaceous corolla, while the latter showed non-papilionaceous corolla. Anthesis occurred predominantly between 8:00-11:00 AM across genotypes. Pollen viability was consistently high (>96%) at anthesis. *in vitro* pollen germination ranged from 79.52% to 91.81%, with pollen tube lengths varying between 1,269.41 µm and 1,546.29 µm. Stigma receptivity was highest at anthesis and gradually declined after 24 hours. Double-petalled types exhibited comparatively lower pollinator visit due to complex floral structure. The study underscores the influence of floral architecture on reproductive success and provides valuable insights for breeding strategies aimed at balancing seed yield with and phytochemical utility.

Keywords: *Clitoria ternatea*, floral biology, pollen viability, stigma receptivity, genetic improvement

Introduction
Clitoria ternatea L. commonly known as shankhpushpi, butterfly pea, blue pea, or aparajita, is a perennial leguminous climber belonging to the family Fabaceae and subfamily Papilionaceae. The genus comprises about 60 species, mostly distributed in tropical regions with a few extending into temperate zones. *C. ternatea* has gained global attention due to its ornamental, medicinal and nutritional values. The vivid blue flowers are a rich source of anthocyanins, which are used as natural food colorants and herbal tea ingredients, and possess antioxidant, antimicrobial and neuroprotective properties (Chusak *et al*. 2018; Vidana *et al*. 2021) [3, 11]. Because of these attributes, the species is now cultivated not only in traditional systems but also in commercial sectors for pharmaceutical and nutraceutical applications.

Despite its growing importance, knowledge of the reproductive biology of *C. ternatea* remains limited. In legumes, reproductive success is closely linked to floral traits such as anthesis timing, pollen viability, stigma receptivity and the efficiency of pollinator-mediated pollen transfer. Both single-and double-petalled floral morphotypes are present in this species, and differences in floral architecture are likely to influence pollinator attraction and effectiveness. Understanding these interactions is particularly important, as successful pollination underpins seed set, genetic improvement, and sustainable cultivation.

Given these gaps, the present study was undertaken to investigate the floral biology, pollen viability, *in vitro* pollen germination, stigma receptivity and pollinator activity in contrasting genotypes of *C. ternatea*. The insights generated are expected to provide a scientific basis for breeding programs targeting both medicinal and ornamental traits, while also contributing to the broader understanding of pollination ecology in leguminous crops.

Materials and Methods
The present investigation was conducted at the ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka.

Twelve contrasting genotypes of *Clitoria ternatea* were used in the study, comprising four single blue-petalled (IIHR-CT-5, IIHR-CT-55, IIHR-CT-78, IIHR-CT-101), four single white-petalled (IIHR-CT-11, IIHR-CT-16, IIHR-CT-20, IIHR-CT-107) and four blue double-petalled types (IIHR-CT-63, IIHR-CT-64, IIHR-CT-67, IIHR-CT-91). All genotypes were raised under uniform field conditions following recommended agronomic practices.

Floral biology was studied by examining the corolla pattern, which was visually classified as papilionaceous (typical of Fabaceae, with a standard, wings and keel) or non-papilionaceous, based on petal arrangement and floral symmetry. Anthesis time was determined by visually monitoring flowers at regular intervals in the morning, afternoon and evening and recording the time of opening in all genotypes.

For pollen studies, pollen viability was assessed at three stages *i.e.*, one day before anthesis, on the day of anthesis and one day after anthesis. Fresh pollen grains were collected and dusted onto clean glass slides, stained with Alexander's stain. The slides were scored under compound microscope (40X), as shown in Figure-1 Replicated data was taken and presented in percentage by using the following formula.

$$\text{Pollen viability\%} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Pollen germination test was done on the day of anthesis using Brew baker and Kwack"s medium (1963), with 10% sucrose concentration. The medium contains 100 mgl^{-1} Boric acid, 300 mgl^{-1} Calcium nitrate, 200 mgl^{-1} Magnesium sulphate, 100 mgl^{-1} Potassium nitrate. Pollen grains were collected from flowers picked randomly at antheis (9-11 A.M) in the morning from 4 blue single petalled, 4 white single petalled and 4 blue double petalled genotypes separately. A drop of pollen germination medium was placed in a cavity slide and pollen was dusted over and covered with cover slip with its periphery sealed with vaseline. After 24 hours of incubation in the humid chamber, germination percentage was recorded under the microscope. Pollen showing tube lengths longer than the pollen diameter were scored as germinated. From each field, total number of pollen grains and the number of germinated pollen grains were recorded.

$$\text{Pollen germination\%} = \frac{\text{Number of germinated pollen per field of view}}{\text{Total number of pollen per field of view}} \times 100$$

Pollen grain size (length and width) on the day of anthesis was measured using compound microscope (40× objective). Pollen tube lengths were also measured under the same setup after 24 hours of incubation in germination medium. Data were expressed as mean values in micro meters (µm).

Stigma receptivity was determined by the hydrogen peroxide (6%) test, (Dafni *et al*., 1998) [4] in which flowers sampled at three stages (one day before anthesis, at anthesis and one day after anthesis) were placed on cavity slides and treated with 6% H_2O_2. The stigma receptivity was evaluated by the method adapted by Makwana *et al*., (2017) [8], as represented in Table-1. Pollinator activity was recorded during peak anthesis (08:00-11:30 AM) for one week in all genotypes by noting the number of flowers visited per plant and the duration of each visit.

Table 1: Scoring pattern of stigma receptivity

Score	Description
+	Less receptivity
+ +	Moderate receptivity
+ + +	High receptivity

Data on pollen viability, germination percentage, pollen tube length, pollen grain size, and flower longevity were subjected to analysis of variance (ANOVA) in a randomized block design (RBD) with three replications in GRAPES software (Gopinath *et al*. 2020) [6].

Results

Corolla architecture and anthesis

The evaluated genotypes were classified into two distinct categories based on corolla morphology: papilionaceous and non-papilionaceous types (Table 2). Single-petalled genotypes, including IIHR-CT-5, IIHR-CT-11, IIHR-CT-16, IIHR-CT-20, IIHR-CT-55, IIHR-CT-78, IIHR-CT-101 and IIHR-CT-107, exhibited a typical papilionaceous corolla with a distinct standard, wings and keel, as commonly observed in Fabaceae members. In contrast, the double-petalled genotypes IIHR-CT-63, IIHR-CT-64, IIHR-CT-67 and IIHR-CT-91 displayed non-papilionaceous floral architecture.

Anthesis was observed exclusively in the morning hours, starting at approximately 8:00 AM and completing by 11:00 AM, across all evaluated genotypes. The timing of anthesis was uniform and showed no variation among genotypes.

Pollen viability

Pollen viability showed significant variation across stages as represented in Figure-2, Table-2 (one day before anthesis, on anthesis, and one day after anthesis). One day before anthesis, viability ranged from 64.05% (IIHR-CT-5) to 100.00% (IIHR-CT-91). Other high values were recorded in IIHR-CT-107 (97.44%) and IIHR-CT-78 (97.33%). Lower values were seen in IIHR-CT-5 (64.05%) and IIHR-CT-11 (73.81%).On the day of anthesis, pollen viability was uniformly high across all genotypes, ranging from 96.39% to 100.00%. The highest viability (100.00%) was observed in IIHR-CT-55, IIHR-CT-64, IIHR-CT-91 and IIHR-CT-107, while the lowest was in IIHR-CT-101 (96.39%).

One day after anthesis, a gradual decline in viability was evident, with values ranging from 76.59% to 100.00%. Retention of viability was higher in IIHR-CT-55 and IIHR-CT-107 (100.00%), followed by IIHR-CT-91 (97.22%) and IIHR-CT-64 (96.97%). The lowest viability was observed in IIHR-CT-11 (76.59%) and IIHR-CT-5 (92.78%).

In-vitro pollen germination

On the day of anthesis, significant variation in pollen germination percentages was recorded across genotypes, ranging from 79.52% to 91.81% (Table 3). The highest germination was noted in IIHR-CT-107 (91.81%), followed by IIHR-CT-67 (90.50%) and IIHR-CT-5 (87.91%). The lowest germination percentage was observed in IIHR-CT-16 (79.52%).

Pollen grain and pollen tube size

Significant variation was observed in pollen grain size across genotypes. Length ranged from 152.19 µm (IIHR-CT-16) to 191.30 µm (IIHR-CT-91), while width varied between 136.37 µm and 177.13 µm (Table 2). Pollen tube length also showed considerable variation (Table 3). The longest pollen tubes were recorded in IIHR-CT-16 (1546.29 µm) and IIHR-CT-107 (1530.32 µm), while the shortest was noted in IIHR-CT-5 (1152.30 µm).

Stigma receptivity

Stigma receptivity varied across genotypes and floral stages (Figure 3). The highest receptivity (+ + +) was observed on the day of anthesis in most genotypes than on one day before and one day after anthesis. Strong and consistent receptivity scores (+ + to + + +) across stages were recorded in IIHR-CT-11, IIHR-CT-55, IIHR-CT-63, IIHR-CT-64, IIHR-CT-91 and IIHR-CT-107.

Pollinator visitation

Pollinator visitation was observed during the morning hours (8:00-11:30 AM), coinciding with anthesis. The major pollinators included *Amegilla spp*. (blue-banded bees), *Bombus spp*. (bumble bees), *Apis florea* (little bee), *Xylocopa spp*. (carpenter bees) and *Syrphidae spp*. (syrphid flies).

Active foraging was recorded only in single-petalled genotypes, irrespective of flower colour (Figure 4). In contrast, double-petalled genotypes (IIHR-CT-63, IIHR-CT-64, IIHR-CT-67 and IIHR-CT-91) did not attract any pollinators during the observation period.

Discussion

The variation in corolla architecture among genotypes of *C. ternatea* highlights two distinct morphotypes. While single-petalled forms exhibit the classical papilionaceous type typical of Fabaceae, double-petalled genotypes deviate markedly from this structure. Similar deviations in floral morphology have been documented in *Lathyrus sativus* under wide hybridization, where modifications in petal arrangement altered floral symmetry and function (Chopkar, 2021) [2]. Such modifications in *C. ternatea* likely influence pollinator accessibility and consequently reproductive efficiency.

Uniform anthesis during morning hours (8-11 AM) indicates that flowering is synchronized with the activity period of major insect pollinators such as bees. This synchrony maximizes pollen transfer and fertilization efficiency, a trait commonly reported in other Fabaceae crops.

Pollen viability peaked on the day of anthesis, confirming that this is the optimal stage for successful fertilization. The decline before and after anthesis is consistent with patterns observed in pigeon pea (Kar & Datta, 2017) [7]. The ability of some genotypes (IIHR-CT-55, IIHR-CT-107) to retain high viability even after anthesis suggests their potential for prolonged reproductive success.

Variation in *in-vitro* pollen germination percentages across genotypes further supports the role of genetic factors in determining reproductive potential. This observation is in agreement with Wasonga (2020) [12], who reported similar variability in *Crotalaria* species. Genotypes such as IIHR-CT-107 and IIHR-CT-67, which exhibited high germination, may be considered reproductively superior.

Differences in pollen grain size and pollen tube length also reflected genotypic diversity. Larger grains and longer pollen tubes, as observed in IIHR-CT-91, IIHR-CT-16 and IIHR-CT-107, are indicative of higher pollen vigour and fertilization efficiency. Smaller pollen grains and shorter tubes, such as in IIHR-CT-5, may limit reproductive capacity. Such morphological and physiological traits are critical determinants of reproductive success in legumes.

Stigma receptivity was highest on the day of anthesis, with many genotypes showing strong receptivity scores (+ + +). This synchrony between pollen viability, germination potential and stigma receptivity ensures maximum chances of successful fertilization. Genotypes such as IIHR-CT-55 and IIHR-CT-107, which showed strong and consistent receptivity, are particularly promising for breeding purposes.

Pollinator observations revealed a clear distinction between single and double petalled morphotypes. Active visitation by little bees and carpenter bees was confined to single-petalled genotypes, while double-petalled types showed no visitation. This absence of pollinators in double-petalled forms may be attributed to structural barriers that hinder access to reproductive organs. Similar findings have been reported earlier in *C. ternatea* by Prafulkumar (2011) [9], Girish Kumar (2017) [5], who documented diverse pollinator species including *Amegilla*, *Xylocopa* and *Papilio*.

Table 2: Floral morphology and Pollen viability across the genotypes

Genotypes	Corolla type	Pollen viability (%)		
		One day before anthesis	On anthesis day	One day after anthesis
IIHR-CT-5	Papilionaceous	64.05±9.59	97.22±4.81	92.78±6.74
IIHR-CT-11	Papilionaceous	73.81±2.06	96.82±2.76	76.59±6.11
IIHR-CT-16	Papilionaceous	91.69±5.21	97.59±2.51	96.34±4.80
IIHR-CT-20	Papilionaceous	93.06±6.36	96.82±2.76	94.25±3.04
IIHR-CT-55	Papilionaceous	95.83±7.22	100.00±0.00	100.00±0.00
IIHR-CT-63	Non-papilionaceous	94.84±4.51	97.92±3.61	95.24±8.25
IIHR-CT-64	Non-papilionaceous	92.13±6.85	100.00±0.00	96.97±5.25
IIHR-CT-67	Non-papilionaceous	95.54±3.89	98.48±2.62	94.44±9.62
IIHR-CT-78	Papilionaceous	97.33±4.62	96.81±2.87	94.66±4.64
IIHR-CT-91	Non-papilionaceous	100.00±0.00	100.00±0.00	97.22±4.81
IIHR-CT-101	Papilionaceous	93.89±5.36	96.39±3.37	94.87±8.88
IIHR-CT-107	Papilionaceous	97.44±4.44	100.00±0.00	100.00±0.00
SE (M)		3.33	1.59	3.16
CD @ 5%		9.78	4.67	9.27
CV		2.81	6.36	5.78

Table 3: Pollen grain and pollen tube sizes and *in vitro* pollen germination percentages in different genotypes on Anthesis day

Genotypes	Pollen grain length (µm)	Pollen grain width (µm)	*In-vitro* pollen germination (%)	Pollen tube length (µm)
IIHR-CT-5	171.46±4.14	148.89±8.84	87.91±4.02	1152.30±135.26
IIHR-CT-11	183.72±14.23	165.10±13.67	85.80±2.83	1395.70±14.97
IIHR-CT-16	171.07±5.10	157.29±11.14	79.52±5.07	1546.29±289.94
IIHR-CT-20	152.19±8.51	137.42±13.08	85.63±10.16	1180.53±33.60
IIHR-CT-55	189.07±5.21	136.37±13.48	81.99±4.55	1336.01±172.97
IIHR-CT-63	171.12±10.03	147.10±15.77	82.39±8.02	1187.01±144.24
IIHR-CT-64	179.57±12.49	147.10±15.77	81.35±5.63	1291.70±49.58
IIHR-CT-67	172.30±8.49	163.91±5.26	90.50±4.32	1440.81±289.33
IIHR-CT-78	163.90±14.96	153.92±9.13	81.44±6.57	1440.24±254.96
IIHR-CT-91	191.30±9.23	177.13±5.96	84.05±4.51	1357.50±139.58
IIHR-CT-101	169.81±15.09	154.20±8.53	84.71±8.52	1212.62±133.45
IIHR-CT-107	188.05±5.99	176.05±11.29	91.81±0.98	1530.32±166.98
SE (M)	5.22	5.62	3.29	128.29
CD @ 5%	15.03	16.18	9.65	9.60
CV	5.96	7.24	6.72	13.55

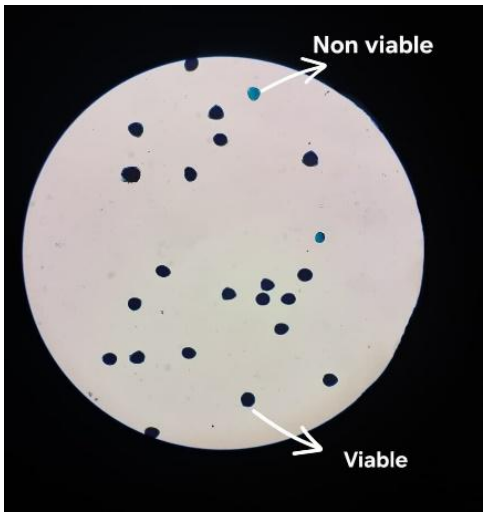

Fig 1: Viability of pollen

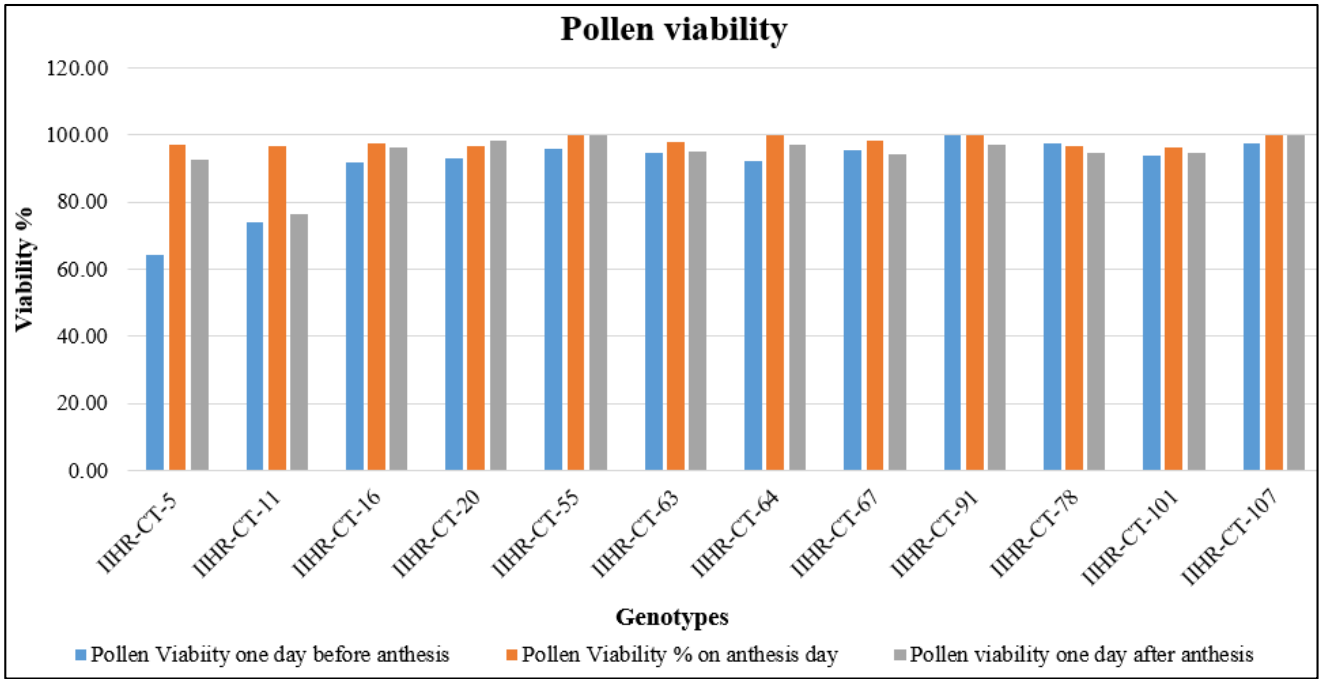

Fig 2: Pollen viability in *Clitoria ternatea* genotypes in various days

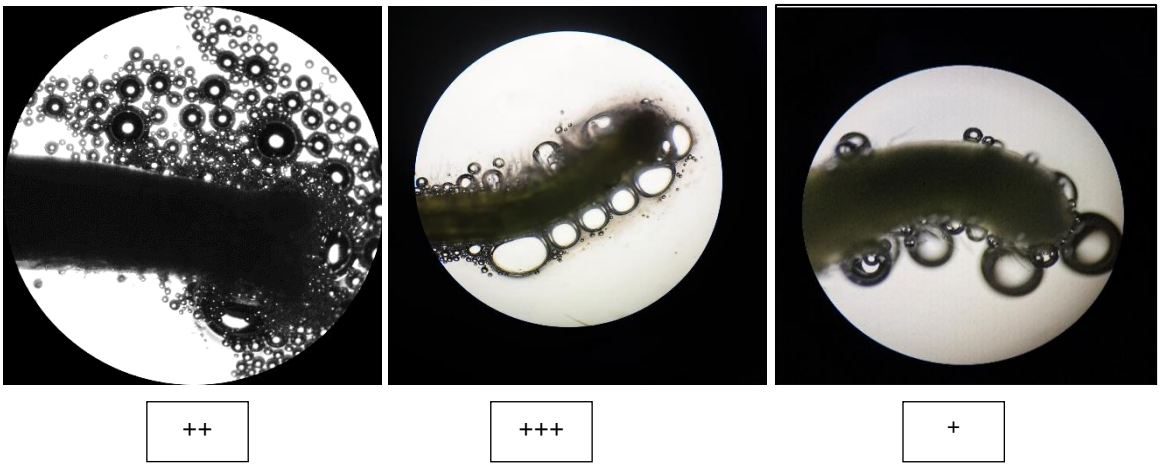

Fig 3: Scoring of stigma

Xylocopa micans in Single petalled blue and white genotypes

*Apis florea in singl*e petalled white and blue genotypes

Fig 4: Pollinators visitation in *Clitoria ternatea*

Conclusion

The present study on *Clitoria ternatea* revealed significant genotypic variation in floral biology, pollen viability, germination and pollen tube growth, all of which play a crucial role in reproductive success. Anthesis occurred uniformly across genotypes, while pollen viability was highest on the day of anthesis, declining gradually thereafter. Although pollen grain size varied considerably, it did not always correspond with pollen tube length, indicating that vigour is determined more by physiological efficiency than by morphology alone. Genotypes such as IIHR-CT-16 and IIHR-CT-107 exhibited superior pollen vigour, suggesting their potential for enhanced fertilization efficiency. The observed differences highlight the importance of combining pollen viability and tube growth assays to assess male gametophytic performance, rather than relying solely on morphological traits. Overall, the findings provide baseline information useful for breeding, hybridization and genetic improvement programmes in *Clitoria ternatea*.

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