

Research Article

Butterfly Pea (*Clitoria ternatea* L.: Fabaceae) and Its Morphological Variations in Bali

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ABSTRACT

Butterfly pea (*Clitoria ternatea* L.) is an important perennial herbaceous plant with a range of uses as ornamental plants, fodder crops, medicine, and sources of natural food colorant and antioxidants. The leaves and pods are commonly used as a source of protein in fodder, while the flowers are usually dried and processed as a high antioxidant-containing tea. The blue variant of butterfly pea was the most commonly used variety, although there are quite diverse butterfly pea varieties. The present study aimed to observe the morphological variations among the 26 butterfly pea accessions that originated from a wide range of areas in Bali. The explorative method was used to obtain diverse specimens (accessions) of butterfly pea in Bali, and subsequently, morphological characterization of the accessions was performed. The primary data of morphological traits that were recorded included stems, leaves, flower structures, flower colors, pods, and seeds. The data were analyzed descriptively to determine the morphological variations between accessions. The results showed three major morphological variations: (i) the colour of the flower (corolla), (ii) the corolla structure, and (iii) the stamen structure. The colour of corolla has four variations: white, mauve, light blue, and dark blue; while the corolla structure has two variations: normal and multiple layered corollas. The stamen character showed a correlation with the structure of the corolla. The normal corolla has diadelphous stamens, while the multiple layered corollas have solitary stamens. These morphological variations are the genetic richness of Indonesia's biodiversity and should be protected and conserved.

Keywords: butterfly pea, corolla abnormality, CYC gene interaction, diadelphous, papilionoid

INTRODUCTION

Butterfly pea (*Clitoria ternatea* L.) is a perennial herbaceous creeping plant that belongs to the Fabaceae family. This plant is mainly distributed in a tropical region that requires high light intensity and is relatively persistent with abiotic stress (Jamil et al. 2018; Oguis et al. 2019). As a typical legume plant, its roots form nodules that play a major role in nitrogen fixation in soil and act as a natural fertilizer in agricultural land (Oguis et al. 2019). Butterfly pea was first cultivated as an ornamental plant and then shifted as fodder for cattle

because of its high protein content (Mahmad et al. 2016; Jamil et al. 2018; Oguis et al. 2019). As fodder, butterfly pea is also used as a short to middle pasture plant (Hall 1985; Mahmad et al. 2016) with high-quality nutrition for cattle and other ruminant livestock (Abreu et al. 2014). Although popular as an ornamental plant and fodder, the latest research of butterfly pea is on the exploration of its bioactive compound, such as the antioxidant and proteinderived bioactive compounds in butterfly pea (Oguis et al. 2019). Cyclotide was an example of a protein-derived bioactive compound that was found almost in every part of butterfly pea and used as a natural pesticide (Nguyen et al. 2011; Oguis et al. 2019).

The flower of the butterfly pea is the most recognizable part of the plant with very attractive colors. The blue colour is the most widespread variation, so the butterfly pea is also known as the blue pea, although there are other colour variations such as mauve and white. The blue butterfly pea contains a specific anthocyanin called delphinidin 3,5',5'-triglucoside which is also known as ternatins (Saito et al. 1985). The anthocyanin content made the flower has been used as a source of natural antioxidant and natural dyes for food and cosmetic (Oguis et al. 2019). Flower extract of butterfly pea shows better colorant properties among other popular natural colorants such as *Garcinia mangostana* peels, *Ardisia colorata* fruits, and *Syzygium cumini* fruits (Azima et al. 2017). For its utilization in food, Pasukamonset et al. (2018) used the extract of blue-flowered butterfly pea to increase polyphenol content in sponge cake without showing any significant physicochemical differences.

Anthocyanin is not the only antioxidant compound in butterfly pea, it also contains other powerful antioxidants, such as flavonoid, phenolic acid, procyanidin, and flavonol glycoside (Jamil et al. 2018). Although it is popular for its antioxidant content, butterfly pea also contains many bioactive compounds, such as tannin, resin, steroid, saponin, triterpenoid, and xanthene (Manjula et al. 2013). Jamil and Pa'ee (2018) listed every bioactive compound in the roots, stems, leaves, and flowers of butterfly pea that are potential as anti-microbial agents.

In Indonesia, butterfly pea is widely distributed. The variability within species is valuable germplasm and should be well-recorded. Those variabilities can be preserved through sustainable use that combined aspects of utilization and conservation for their long-term utilization and persistence. Butterfly pea is much used as ornamental plants and worshipping materials in Indonesia. In Bali, the demand for flowers as worshipping materials in Hindu ceremonies was high. The people's preference to use local flowers as worshipping materials might unpremeditatedly conserve the existence of butterfly pea variations. The present study aimed to document and record the variability of butterfly pea in Bali based on morphological characterization.

MATERIALS AND METHODS

Study area

The explorative method was used to observe the morphological variation of butterfly pea in Bali. The research was conducted from April to November 2019 in ten areas in Bali that were marked with GPS as shown in Figure 1. The locations and coordinates of each area are listed in Table 1.

Study Species

Butterfly pea grows at 1–700 m above sea level, especially at the dry area on the edge of the road and bush forest (Backer & van den Brink 1963). In this research, the range of its habitat was wider, from dry to wet area (edge of rice field). Most of the butterfly peas were found in the residential area as an

Table 1. Locations of butterfly pea in Bali.

Number	Locations	District, Regency	Latitude
1.	Pekutatan	Jembrana	S: 08°28'32,3" E: 114°50'24,8"
2.	Kayu Putih	Buleleng	S: 08°15′33,1″ E: 115°01′20,4″
3.	Angseri	Tabanan	S: 08°21′49,6″ E: 115°10′11,2″
4.	Buahan Kelod	Gianyar	S: 08°24'10,0" E: 115°14'13,1"
5.	Rendang	Karangasem	S: 08°25′58,3″ E: 115°25′38,9″
6.	Seraya	Karangasem	S: 08°27′31,8″ E: 115°38′11,7″
7.	Kenderan	Gianyar	S: 08°28'14,3" E: 115°17'08,8"
8.	Ubud	Gianyar	S: 08°31′22,1″ E: 115°16′04,4″
9.	Masceti	Gianyar	S: 08°35′30,5″ E: 115°20′46,8″
10.	Jimbaran	Badung	S: 08°47′30,1″ E: 115°10′45,2″



Figure 1. The study site of butterfly pea observation. The numbers indicate the study site: (1) Pekutatan; (2) Kayu Putih; (3) Angseri; (4) Buahan Kelod; (5) Rendang; (6) Seraya; (7) Kenderan; (8) Ubud; (9) Masceti; (10) Jimbaran.

ornamental plant (Figure 2a) or as a worshiping material in Hindu ceremonies (Figure 2b). The adult butterfly pea (>1.5 m in height) produces many flowers that bloom every day. It makes this plant suitable both as an ornamental plant and as a source of worshiping material.



Figure 2. The use of butterfly pea (a) as an ornamental plant on sidewalks; and (b) white butterfly pea as a praying material.

In the scope of taxonomy, butterfly pea was grouped in Leguminosae. Leguminosae is a *nomina conservanda* of the Fabaceae family that has three subfamilies based on its flower morphology: Caesalpinioideae (peacock flower), Mimosoideae (sensitive plant), and Papilionoideae/Faboideae (pea or butterfly flower). Among those three, Papilionoideae was the subfamily showing more advanced evolutionary characteristics (Ojeda et al. 2009). The complete classification of butterfly pea was shown below:

Kingdom	: Plantae		
Division	: Magnoliophyta		
Class	: Magnoliopsida		
Order	: Fabales		
Family	: Fabaceae		
Subfamily	7 : Papilionoideae		
Genus	: Clitoria		
Species	: Clitoria ternatea L.		
Synonym	n :		
	Clitoria albiflora Mattei; C. bracteata Poir.; C. coelestris Siebert		
	& Voss; C. parviflora Raf.; C. philippensis Perr.; C. pilosula		
	Benth.; C. ternatensium Crantz; Lathyrus spectabilis Forssk.;		
	Ternatea ternatea (L.) Kuntze; T. vulgaris Kunth (all derived		
	from The Plant List website at http://		
	www.theplantlist.org/tpl1.1/record/ild-2539)		
Common	name :		
	Butterfly pea, blue pea (English); kembang telang		
	(Indonesia); bunga telang, bunga celeng (Bali)		

Methods

The in-site observation was performed to record the coordinates, habitats, sources (wild or planted), utilizations, and morphological variations. Purposive exploration was conducted around the marked coordinates. The coordinates were marked using GPS Garmin 64csx, then visualized in software QGIS 3.12.3 with base map derived from tanahair.indonesia.go.id.

Twenty-six specimens (accessions) of butterfly pea were characterized directly in the field. The morphological characterization was conducted through the description and measurement of vegetative organs (roots, stems, and leaves) and generative organs (flowers, fruits, and seeds) (Tjitrosoepomo 1985; Rugayah et al. 2004; Bishoyi & Geetha 2013). The description was compared with the main reference *Flora of Java vol. I* (Backer & van den Brink 1963). The morphological characterization was completed with the specimen's documentation by taking the photographs.

The root sampling was performed selectively to get the characteristic of nodules. The roots were sampled from eight plants. The root nodules were sectioned using a cutter knife to expose the infection region, then observed using a magnifier glass. The data of morphological variations were analyzed descriptively as a single description. To preserve the germplasm, the seeds of mature pods were collected. Those seed collections were then deposited at Tropical Forage Research and Development Center in Universitas Udayana, Bali.

RESULTS AND DISCUSSION

Morphological variability of the vegetative organs in butterfly pea (*Clitoria ternatea* L.)

Results of the present study showed there was no morphological variation between the 26 butterfly pea accessions. It appears that roots (including root nodules), stems, and leaves of the 26 accessions of butterfly pea had similar morphology. The 26 accessions had root nodules that were composed of large and small nodules with a round to irregular shape with a size that ranged from 0.5–1.5 cm in diameter, cortex thickness ranged from 0.05–0.1 cm, and infection region up to 90% of the size of the nodule (Figure 3a-c). The distribution of the large and small nodules accords with the research by Skerman et al. (1988). The large nodules distributed sparsely, while the small nodules more clumped or close to each other.

Root nodule is an important specialised organ as the place of the symbiotic association between butterfly pea and nitrogen-fixing bacteria such as *Rhizobium*. At the inner part of the nodule, there is the red part that contains leghemoglobin, a hemeprotein in legume which is responsible to provide oxygen for *Rhizobium* and protect the nitrogenase enzyme complex from irreversible inactivation if exposed to the atmospheric level of oxygen (Singh & Varma 2017). The symbiosis and activity of nitrogen-fixing bacteria in butterfly pea roots made this plant able to grow in an area with low nutrients and at the same time recover the soil fertility in that area (Hall 1985).



Figure 3. Characteristics of root nodules in *Clitoria ternatea* (a) large-sized nodule; (b) small-sized nodule; and (c) section of nodule showing cortex (pale brown) and medulla or infection regions (brown). Scale bar: 0.5 cm.

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Figure 4. Scheme of evolutionary theory in Fabaceae (Skerman et al. 1988).

Based on the root-nodule symbiosis, butterfly pea (*Clitoria ternatea*) that belongs to Papilionoideae can be classified as a more advanced species in evolution compared to other species from other subfamilies within Fabaceae family (Caesalpinioideae and Mimosoideae). In the evolutionary approach of Fabaceae based on the symbiotic relationship, the subfamilies that have a more complex symbiosis with *Rhizobium* are classified as more advanced subfamilies as they usually develop more advanced structures. Skerman et al. (1988) constructed the evolution of Fabaceae as a scheme in Figure 4 and stated that Papilionoideae was the subfamily that was more advanced in evolution based on its high symbiotic relationship.

The stem of the 26 accessions of the butterfly pea in the present study did not show any differences, all was creeping to other plants in the competition of sunlight. The length of the stem reached 5 m, matching with the description from Backer & van den Brink (1963) who stated the length of stems ranged between 1 to 5 m. The stem was woody and green with lignified brown at the base. The green part has public entry white along the stem.

Butterfly pea accessions in the present study had alternate compound leaves, length of peduncle 6–10.5 cm, 5 and 7 leaflets, and opposite phyllotaxis. Leaf-blade ovate–oval, sized $1.5-4.7 \ge 1-3.6$ cm, entire margin, rounded at the base, retuse apex, green at the adaxial surface and paler at abaxial, linear trichomes at both sides of the blade. The terminal leaflet is usually bigger, while the base leaflet is smaller (Figure 5). Peduncle, pedicle, and vein had trichomes as same as stem. Leaflets had 2 linear stipules lengths of 0.2–0.3 cm. Each stem node had a spear-like stipule, 0.2–0.4 \ge 0.1 cm in size.



Figure 5. Compound leaves of butterfly pea with a bigger terminal leaflet for 5-leaflet and smaller terminal leaflet for 7-leaflet. Scale bar: 3 cm.

Morphological variability of the generative organs in butterfly pea (*Clitoria ternatea* L.)

The generative organs of butterfly pea consist of flowers, fruits, and seeds. The present study showed the flower of the butterfly pea was the most variable organ and easily recognized. The variable parts were the colour and structure of corollas (Figure 6a–h) and stamens (Figure 8), while the other parts such as pistils, fruits/pods, and seeds did not show any qualitative variability (Figure 7 and 9).

The flower parts of the butterfly pea consist of bractea, corolla, calyx, pistil, and stamens. Bractea rounded–ovate, $0.5-0.7 \ge 0.5-0.6$ cm in size, entire margin, apex rounded–acute, light green color, pubescent white at both surfaces.

The corolla of butterfly pea is a typical Papilionoideae corolla. Five petals of corolla consist of one petal (the biggest) at anterior called standard or vexillum, two petals at lateral called wings or alae, and two petals at posterior called keels or carina. Vexillum ovoid, 3.5-4 x 4-5 cm, margin entire, acute at the base, apex retuse. Alae semi-circular, 2-3 x 1.1-1.5 cm, elongated at the base, from the middle to terminal overlapped one another. Carina semi-circular, 1-2 x 0.3-0.5 cm, elongated at the base and attached to the receptacle, concave at the middle to protect the pistil and stamens. In some accessions, half part of the carina is attached to the alae. Variation in the structure of corolla in the present study is distinguished as (i) normal corolla and (ii) multiple layered corollas (Figure 6). The normal corolla was the typical Papilionoideae corolla described above, while the multiple layered corollas were the mutant's petal variation. The wings and keels in mutants were bigger and even attaining the size of a vexillum (Bishoyi & Geetha 2013). It leads to the shift of the flower symmetry from bilateral symmetry that is typical in papilinoid flower to be radial symmetry (Bishovi & Geetha 2013).

Fu et al. (2005) stated that the character of corolla was determined by two genes *CYC2* (*CYCLOIDEA 2*) which act as a transcription factor for TCP gene complex to code vexillum and *CYC3* (*CYCLOIDEA 3*) that influence the character of alae. The appearance of multiple corollas in butterfly pea could be caused by mutation or crossing over that involved in



Figure 6. Variation in the corolla structure of the butterfly pea (a) the comparison of the whole corolla in normal (left) and multiple layered corollas (right); (b) Normal corolla (left), multiple layered corollas (right). The parts of normal corolla are carinas or keels (i), wings (ii), and vexillum (iii). The difference between the normal and multiple layered corollas is in the size of keels and wings relative to the size of the vexillum. The carinas, wings, and vexillum in multiple layered corollas also had recurved to revoluted margin and base. Scale bar: 3 cm.

over-expression of *CYC2* gene and induce all of the petals shaped and sized as vexillum (Figure 7). Wang et al. (2008) also reported the ortholog gene of *CYC2* and *CYC3* also played a vital role in the zigomorphism of the *Pisum sativum* flower. Those gene interactions indicate that the genetic regulation was responsible to determine flower characteristics in Papilionoideae.

The colour of corolla of the butterfly pea accessions in the present study has four variations: white, mauve, light blue, and dark blue, which were found in both normal and multiple layered corollas (Figure 7a–h). The variations of butterfly pea corolla colour in the present study were similar to Backer & van den Brink (1963), Reid & Sinclair (1980), Morris (2009), and Backer & van den Brink (1963) that stated that the corolla of butterfly pea was dark blue, mauve, or white with yellow in the middle and white spot at the edge. The differentiation of colour in corolla is caused by the secondary metabolites anthocyanin. In butterfly pea, the mauve and blue colour are caused by anthocyanin content such as delphinidins, while the white colour did not contain anthocyanin (Kazuma et al. 2003).

The variation in corolla and colour structure of butterfly pea in the present study might be related to genetic factors. Cronk (2006) stated that gene interactions can lead to the variation in shape, color, and epidermal type of corolla.



Figure 7. Characteristic of structure and colour of butterfly pea corolla (a) normal white corolla; (b) normal mauve corolla; (c normal light blue corolla; (d) normal dark blue corolla; (e) multiple layered white flower; (f) multiple layered mauve flower; (g) multiple layered light blue corolla; and (h) multiple layered dark blue corolla. Scale bar: 3 cm.

The calyx of the butterfly pea in the present study did not show any variation. The calyx consisted of five sepals that adnate from the base to the middle (partitus). Sepal was green, pubescent white, and forming calyx-tube with length 1.5–1.7 cm. In dry areas, the colour of sepals tends to purplish or reddish.

The present study also showed variation in stamen structure which was related to the variation of corolla structure (normal and multiple layered corollas). Although both normal and multiple layered corollas had 10 stamens (Figure 8), the stamens in normal corolla were arranged in a diadelphous structure with 9 adnate stamens and 1 solitary stamen below the pistil as same as recorded by Backer & van den Brink (1963). Adnate stamen length 1.3 cm continued with 0.5 cm filament that supports each anther. In multiple layered corollas, all those 10 stamens were arranged solitarily around the pistil with straight or curly filaments 1.8–2.0 cm in length.



Figure 8. Stamen variation of butterfly pea. Diadelphous stamens (left) and solitary stamens (right). Scale bar: 0.5 cm.

In diadelphous, all of the stamens were located around the pistil, while in solitary stamen was not. The revoluted base of the keels and wings in multiple layered corollas isolated 2–4 solitary stamens from the pistil. It affected the quantity of the anthers around the stigma. If compared with diadelphous with 10 anthers around the stigma, the solitary stamens only had 6-8 anthers around the stigma. For pistil, there was no qualitative variation between the 26 accessions of the butterfly pea in the present study. The stylus was green and thick, 2–2.2 cm in length, the stigma square-shaped, green, and covered with dense white trichomes that have a role to catch the pollens.

Pollination occurs when pollen was attached to stigma, then continued to form pods. The pods of the 26 accessions of butterfly pea in the present study had an elongated oval shape, $7-10 \ge 1$ cm, dried calyx, and bractea at the base, apex caudate with 1-1.5 cm length, green when young and turn brown at maturity, pod cracked at the middle and then rolled up (Figure 9a). The seeds were brown-black with white elaiosome, cylindrical-square or reniform, $1 \ge 0.4$ cm (Figure 9b). Each pod contains 3-10 seeds.

Bali has eight morphological varieties of butterfly pea. In annotations, the normal-light blue and the normal mauve were the rarest variety in Bali. Morris (2009) stated that morphological variations in butterfly pea are related

to its diverse geographic origins. The eight morphological variabilities can be used to compose the identification key of the butterfly pea at the level of intraspecific as shown below.

- - b. The corolla colour is mauve or light mauve normal-mauve variant
 - c. The corolla colour is light blue normal-light blue variant
- d. The corolla colour is dark blue normal-dark blue variant
- - d. The corolla colour is dark blue multiple layered-dark blue variant
 - d. The corolla colour is dark blue multiple layered-dark blue valiant

Based on its potential and variability, butterfly pea has a high value as important germplasm in Indonesia that can be utilized as fodder, natural dye for clothes and food, and as a source of bioactive compounds. In Australia, butterfly pea has been developed as a superior fodder by cultivar selection that is known as 'Milgarra' (Conway & Doughton 2005). The use of butterfly pea in Indonesia is not as massive as in Australia, even though the climate in Indonesia is suitable for butterfly pea cultivation.

A Conservation approach is required in the utilization of butterfly pea in the scheme of sustainable use combining aspects of conservation and utilization in a balance for the long-term survival and long-term utilization of the butterfly pea. Furthermore, research in a wide scope on butterfly pea is required for the sustainable use of the butterfly pea. Mahmad et al. (2016) developed an artificial seed of butterfly pea from its callus to decrease the exploitation in the wild. The research in the scope of ecology, physiology, biochemistry, cytology, and molecular is required to support the conservation and sustainable use of butterfly pea.



Figure 9. The characteristics of pods and seeds (a) development of the pods from the green pods (left) to the brown pods (right). The mature pods are usually hidden under the newly sprout leaves; and (b) seeds. Scale bar: 1 cm (a) and 0.5 cm (b).

CONCLUSION

Butterfly pea (*Clitoria ternatea* L.) has three major variations (i) the corolla color, (ii) the corolla structure, and (iii) the stamen structure. The colour of

corolla has four variations: white, mauve, light blue, and dark blue, while the corolla structure has two variations: normal and multiple layered corolla. The stamen morphology is related to the corolla structure. The normal corolla has diadelphous stamens, while the multiple layered corollas has 10 solitary stamens. These variations were the potential germplasm that should be protected and conserved.

AUTHORS CONTRIBUTION

I.W.S. designed the research, collected the data, and wrote the manuscript. I.M.S.W. collected and analyzed the data and wrote the manuscript.

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CONFLICT OF INTEREST

We have no conflict of interest regarding the research or the research funding.

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