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Morphological characterization and assessment of anthocyanin in three different genotypes of *Clitoria ternatea* L

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Abstract
Clitoria ternatea L. commonly called as Bluepea is a tropical flower, that has been used worldwide as an ornamental flower, and is traditionally used as natural food colorant benefiting the blue color, that is due to anthocyanin compounds. Anthocyanin has been identified as a functional molecule because of its antioxidant potential. It has two major flower colors viz., blue and white. Blue single layered, blue double-layered, and White single layered seeds were collected and sown to observe their morphological characterization and for anthocyanin assessment. Based on the study, it was observed that blue flowers possess anthocyanin but the highest was found in Double layered flowers (51.90 mg/l). Blue double layered showed higher plant height on measuring at 60 DAS, larger flower length, wider width, heavier flower weight, and higher total phenol content (38.92 mg GAE/g).white flower produced longer leaf length and pod length, wider pod width, heavier pod weight. The blue single produced heavier leaf weight, seed weight and the highest total chlorophyll (2.58 mg/g).

Keywords: Blue pea, *Clitoria*, morphology, anthocyanin, phenols

1. Introduction
Blue pea (*Clitoria ternatea* L.) is a perennial herbaceous plant belongs to the Fabaceae family, mostly distributed in a tropical region that needs high light intensity and is relatively persistent with abiotic stress (Jamil *et al*., 2018) [4]. It produces solitary, axillary, and papilionaceous flowers containing five petals, including a standard petal, two keel parts, and two wing petals (Bishoyi & Geetha, 2012) [3]. Blue pea was first cultivated as an ornamental plant (Mahmad *et al*., 2016) [9]. Although blue pea is popular as an ornamental plant and feed, recent research has focused on the search for bioactive compounds such as antioxidant and protein-derived bioactive compounds in blue pea (Oguis *et al*., 2019) [11]. Blue pea flowers are the most recognizable part of the plant with a very attractive color. Since blue color is the most spread variation, so *Clitoria ternatea* is also commonly called bluepea, also there are other color variations such as purple and white (Saito *et al*. 1985) [13]. In blue pea, anthocyanin is not the only antioxidant but also contains other powerful antioxidants, such as phenolic acid, flavonoid, flavonol glycoside, and procyanidin (Jamil *et al*., 2018) [4]. The anthocyanin content in blue pea flowers has been used as a source of natural colorants and natural antioxidants in foods and cosmetics (Oguis *et al*. 2019) [11]. Several pharmacological effects like antimicrobial, antidiabetic, anticancer, anti-inflammatory was reported (Al-snafi, 2016) [1]and (Havananda and luengwilai, 2019) [16]. In recent days, *Clitoria* has attracted lot of interest because of its wide use. *Clitoria* is rich source of anthocyanin and phytochemical constituents in its plant parts. It has potential use in food, nutraceutical industry, but no crop improvement research is reported. Knowledge of variability among genotypes is necessary for the improvement of crop. On this requirement, the study was taken up to observe variation in morphology, total chlorophyll, anthocyanin, and phenol content among 3- different genotypes of *Clitoria* (Blue single, blue double-layered, and white single).

2. Materials and Methods
The study was carried out with 3 genotypes of *Clitoria ternatea* at the Department of Floriculture and Landscape Architecture, Tamil Nadu Agriculture University, Coimbatore in Completely Randomized Design. The seeds were collected from places in and around Coimbatore and sown. Five month old plants were selected and observations was recorded on traits like plant height, leaf length, leaf width, leaf weight, petal arrangements, flower length,

flower width, flower weight, 5 stages of flower development, pod length, pod width, pod weight, seed weight, and total chlorophyll, total anthocyanin content, total phenol content. The results were statistically analyzed using AGRES software package and MS Excel® spreadsheet.

Fig 1: *C. ternatea* Blue (single-layered flower)

Fig 2: *C. ternatea* Blue (Double-layered flower)

Fig 3: *C. ternatea* White (single-layered flower)

2.1 Preparation of *Clitoria ternatea* flowers

After harvesting, flowers were dried under shade till it reaches 5-6% moisture, was packed in dark bags, and stored at ambient temperature (28 ºC ± 2 ºC) for further use. The ratio of raw material (butterfly pea dried flower) to solvent (water) was 1:10 (*w/v*). Extraction was started by mixing 1 g of dried *Clitoria* flowers in 10 ml of distilled water, with an extraction temperature 70 ºC. The samples were then centrifuged for 30 minutes (Nguyen et al., 2021) [10].

2.2 Total Monomeric anthocyanin

Monomeric anthocyanin content was measured using a pH differential method. The extracts were mixed with 0.025 M potassium chloride adjusted to pH 1 and sodium acetate buffer of pH 4.5 separately and then measured at the same wavelength (Lee *et al.*, 2005) [7] against distilled water as blank. The anthocyanin content was calculated using the formula mentioned below and the results were expressed as cyanidin-3-glucoside equivalent in mg/l.

$$\text{Total monomeric anthocyanin } (mg/l \text{ c 3 g eq.}) = \frac{(Aabs * MWt * DF * 103)}{(e * l)}$$

Where,
Abs (Absorbance) = (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5
MWt (Molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu)
DF = Dilution factor
e = Molar extinction coefficient of cyanidin-3-glucoside (26 900 L mol $^{-}$1 cm^{-}1)
l = Path length of cuvette in cm
10^3 = Conversion Factor (g to mg)

2.3 Estimation of total phenol content

Total phenol content was estimated based on Folin–Ciocalteu (FC) method [15] with slight modification. 0.2 ml of the extract was diluted with 8.5 ml of water to which 0.5 ml of Folin–Ciocalteu (FC) was added and incubated for 3 minutes. Then 1 ml of sodium carbonate (20%, w/v) was added. The solution was mixed well and incubated at room temperature for 60 minutes. After incubation, the absorbance was measured at 760nm. A suitable calibration curve was prepared using gallic acid and the results are expressed in milligram per gram (mg/g) gallic acid equivalent.

2.4 Extraction and estimation of Chlorophyll content

250mg of finely cut fresh leaves were taken and macerated with 80% acetone. Then this extract was centrifuged at 3000 rpm. The supernatant was transferred and made up to the volume of 25 ml using 80% acetone. The absorbance was measured at 645 and 663 nm using a spectrophotometer. The chlorophyll content of the samples was calculated using the formula mentioned in (Yoshida *et al*., 1971) [18] and expressed as mg g-1 of fresh leaf.
Chlorophyll a = [12.7(A663) – 2.69 (A645)]*V/1000*W
Chlorophyll b = [22.9(A645) – 4.68 (A663)]*V/1000*W
Total Chlorophyll = [20.2 (A645) + 8.02(A663)] *V/1000*W
Chlorophyll a b ratio = Chlorophyll a / Chlorophyll b
Where
A = Absorbance of specific wavelength
V = Final volume of Chlorophyll extract in 80% Acetone
W = Weight of leaf sample in gram.

3. Result and Discussion

3.1 Morphological variability between 3- different genotypes of *Clitoria ternatea*

Table 1 presents the plant height at 60 DAS, leaf length, leaf width, and leaf weight measurement of 3 different genotypes of *Clitoria*. Taller plant height was attained by Blue double (86.42 cm) followed by white single and blue single, which was statistically comparable ranging from 53-95cm. These findings were reported by (Udai Pal Singh *et al*., 1997) [14]. The leaf length was higher in white single (3.62 cm) followed by blue double and blue single. Wider leaf width was observed in blue double (2.35 cm) followed by Blue single and white single. These results are in association with studies by (Lorelyn Joy *et al*., 2021) [8]. The leaf weight was highest in blue single (96.62mg) followed by white single and blue double. In spite of shorter length, blue single produced heavier weight which may be due to thickness and high moisture content, Similar results were reported by (Komaraiah Karnati *et al.*, 2010) [6].

Table 1: Morphological variation in plant height and leaves of 3-different genotypes of *Clitoria*

Genotype	Plant height (cm) 60 DAS	Leaf length (cm)	Leaf width (cm)	Leaf weight (mg)
Blue single	65.16	3.01	2.15	96.62
Blue double	86.42	3.55	2.35	85.35
White single	74.22	3.62	2.08	91.47
Mean	75.26	3.39	2.19	91.14
SE.d	4.23	0.11	0.25	1.05
CD (P=0.05)	9.21	0.25	0.55	2.28
	S	S	NS	S

S-Significant, NS- Not significant

Table 2 presents the flower length, flower width, and flower weight measurement of 3 different genotypes of *Clitoria* at maturity stage. Longest flower length(4.57cm), wider flower width(3.25cm) and highest flower weight(453.45mg) was observed in blue double which is a potential ornamental followed by blue single, white single. These results are in association with studies by (Lorelyn Joy *et al*., 2021) [8].

Table 2: Morphological variation in flowers of 3-different genotypes of *Clitoria*

Genotype	Flower length (cm)	Flower width (cm)	Flower weight (mg)
Blue single	4.45	3.03	324.15
Blue double	4.57	3.25	453.45
White single	4.40	2.48	264.20
Mean	4.47	2.92	347.26
SE.d	0.10	0.09	7.58
CD (P=0.05)	0.22	0.20	16.52
	NS	S	S

S-Significant, NS- Not significant

Table 3 represents the size measurement in five flower developmental stages of 3 different genotypes of *Clitoria*. On all 5 different stages of flower development, blue double flower showed longer flower length of (1.45cm, 1.83cm, 2.51cm, 2.86cm, 4.52cm at stage-1,2,3,4,5 respectively) and wider flower width of (0.64cm, 0.73cm, 0.85cm, 0.97cm, 3.32cm at stage- 1,2,3,4,5 respectively) followed by blue single and white single. These results are in association with studies by (Kitti Bodhipadma *et al.*, 2017) [5] and (Lorelyn Joy *et al*., 2021) [8].

Table 3: Five developmental stages of *Clitoria* buds to flowers in 3-different genotypes of *Clitoria*

Stages	Blue single		Blue Double		White single	
	Flower length (cm)	Flower width (cm)	Flower length (cm)	Flower width (cm)	Flower length (cm)	Flower width (cm)
1	1.40±0.12	0.52±0.01	1.45±0.14	0.64±0.01	1.13±0.07	0.50±0.01
2	1.80±0.04	0.65±0.02	1.83±0.04	0.73±0.04	1.72±0.03	0.60±0.02
3	2.25±0.05	0.77±0.02	2.51±0.05	0.85±0.02	2.15±0.05	0.73±0.01
4	2.59±0.07	0.93±0.04	2.86±0.06	0.97±0.05	2.50±0.03	0.75±0.05
5	4.44±0.13	2.98±0.06	4.52±0.15	3.32±0.08	4.38±0.11	2.45±0.09

Values were expressed as Mean ± SD (n=5)

Table 4 presents the pod length, pod width, pod weight, and seed weight of 3 different genotypes of *Clitoria* at maturity stage. Longest pod length(9.10cm), wider pod width(0.86cm), highest pod weight(810.78mg) was attained by white single, In spite of its low seed weight than blue single it gained heavier pod weight which may be due to high number of seeds per pod followed by blue single and blue double. Similar findings were reported by (Lorelyn Joy *et al*., 2021) [8].

Table 4: Morphological variation in pods and seeds of 3-different genotypes of *Clitoria*

Genotype	Pod length (cm)	Pod width (cm)	Pod weight (mg)	Seed weight (mg)
Blue single	8.65	0.84	721.26	63.60
Blue double	7.75	0.82	688.10	53.75
White single	9.10	0.86	810.78	62.35
Mean	8.5	0.84	740.04	59.9
SE.d	0.23	0.01	40.78	2.91
CD (P=0.05)	0.51	0.02	88.85	6.34
	S	S	S	NS

S-Significant, NS- Not significant

3.2 Chlorophyll content

Table 5 represents chlorophyll a, chlorophyll b, and total chlorophyll content of 3 different genotypes of *Clitoria*. It was observed that highest chlorophyll-a containing genotype was blue double (1.81mg/g) followed by white single and blue single. Highest chlorophyll-b was noted in blue single (0.75mg/g) followed by blue double and white single. The total chlorophyll was observed high in blue single (2.58mg/g) this may be one reason for heavier leaf weight in blue single, followed by blue double and white single. These results are in association with studies by (Bhavya Doddavarapu *et al.*, 2021) [2].

Table 5: Chlorophyll-A, chlorophyll-B, total chlorophyll pigment quantity of 3-different genotypes of *Clitoria*

Genotype	Chlorophyll a (mg/g tissue)	Chlorophyll b (mg/g tissue)	Total Chlorophyll (mg/g tissue)
Blue single	0.90	0.75	2.58
Blue double	1.81	0.65	2.50
White single	1.62	0.58	2.20
Mean	1.44	0.66	2.42
SE.d	0.03	0.01	0.05
CD (P=0.05)	0.08	0.03	0.12
	S	S	S

S-Significant

3.3 Total anthocyanin content and total phenol content

Table 6 presents the total monomeric anthocyanin and total phenol content from aqueous extract of 3 different genotypes of *Clitoria*. Total monomeric anthocyanin was found high in blue double petal (51.90mg/l) followed by blue single petal (37.17mg/l) lack of anthocyanin results in white single petal. This shows total anthocyanin content was proportional to petal color in different *Clitoria* genotypes. The similar findings were reported by (Tee Havananda *et al.*, 2019) [16]. Total phenol content was calculated on the basis of gallic acid

standard curve, TPC were observed high in blue double petal (38.92mg GAE/g) followed by blue single petal (35.91mg GAE/g) and white single petal (30.16mg GAE/g). Similar findings have been reported by (Lakshan *et al.*, 2020) [12].

Table 6: Total monomeric anthocyanin and total phenol content in flowers of 3-different genotypes of *Clitoria*

Genotype	Total Anthocyanin content (mg/l)	Total phenol (mg GAE/g)
Blue single	37.17±0.35	35.91±0.21
Blue double	51.90±0.56	38.92±1.40
White single	0.00	30.16±0.49

Values were expressed as Mean ± SD (n=3)

(a) (b) (c)

Fig 4: Five developmental stages of *Clitoria* buds to flowers (a) Blue single, (b) Blue Double, (c) White single.

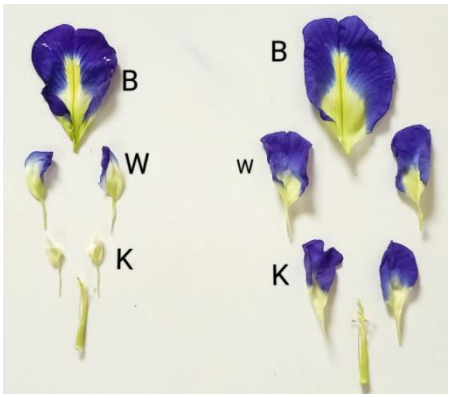

Fig 5: Variation in corolla structure of the *Clitoria*; Blue single-layered (left), Double-layered (right). B is banner, W is wings, K is keels. The difference between single and double layered corolla is in size of keels and wings. The wings and keels in double layered corolla had enlarged.

4. Conclusion

Based on the results of the study, it can be concluded that different genotypes in *Clitoria* also varied with their morphological characteristics, total anthocyanin, and total phenol content. Blue double-layered genotype was good in terms of flowers, plant height, total anthocyanin, and total phenol content. Single-layered genotypes, especially white single was good in terms of pods and seeds. So selection of genotypes should be based on intended use of plant. These potential germplasm that should be protected and conserved.

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