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Genetic characterization of butterfly pea (*Clitoria ternatea*) with different flower colors and petal types using SRAP markers

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Abstract. *Wati SI, Samanhudi S, Yuniastuti E, Purwanto E. 2025. Genetic characterization of butterfly pea* (Clitoria ternatea) *with different flower colors and petal types using SRAP markers. Biodiversitas 26: 3723-3731.* The butterfly pea (*Clitoria ternatea*) is an essential medicinal and ornamental plant in Indonesia, known for its diverse petal colors and forms. This study aimed to assess genetic diversity among eight *C. ternatea* accessions with varying flower colors and petal types using 10 SRAP primer combinations. The 114 DNA bands were amplified, and the average degree of polymorphism across all primer combinations was 87.12%. Two primer combinations (Me3Em3 and Me4Em4) displayed 100% polymorphism. The markers showed an average Polymorphic Information Content (PIC) of 0.31, while the mean Resolving Power (RP) and Marker Index (MI) were 5.50 and 3.24, respectively. The genetic relationships using UPGMA and PCoA revealed two main groups: Cluster 1, which included 1U (purple flower, single petal), 1Pn (pink flower, single petal), 2U (purple flower, double petal), and 2Pn (pink flower, double petal), and Cluster 2, which included 1B (blue flower, single petal), 1Pt (white flower, single petal), 2B (blue flower, double petal), and 2Pt (white flower, double petal). STRUCTURE analysis revealed a more precise genetic separation, with accessions 1U, 1B, 1Pn, and 1Pt clustered in Cluster 1, and 2U, 2B, 2Pn, and 2Pt clustered in Cluster 2. These findings are not only important for breeding programs, but also for conservation strategies. They provide valuable insights that can guide efforts to preserve the genetic diversity of *C. ternatea*, highlighting the crucial role of this research in the broader context of plant conservation.

Keywords: *Clitoria ternatea*, flower, genetic diversity, molecular, petal, SRAP markers

INTRODUCTION

The butterfly pea (*Clitoria ternatea* L.) is a plant that remains underutilized despite its well-recognized potential in various communities (Afrianto et al. 2020, 2021) remains underutilized. This underutilization is significant as it hampers the potential benefits that could be derived from this species. *C. ternatea* is widely utilized, with its flowers for natural dyes and herbal drinks, seeds as animal feed, and roots and leaves for traditional medicine (Afrianto et al. 2020). It is native to Cape Verde, the Arabian Peninsula, and tropical and southern regions of Africa (POWO 2025), and has been widely distributed across tropical and subtropical countries, including Indonesia. *C. ternatea* exhibits diverse flower colors, such as white, pink, blue, and purple (Handayani et al. 2024).

As a perennial and creeping herbaceous plant within the Fabaceae family (Suarna and Wijaya 2021), it thrives in tropical regions with high light intensity and demonstrates considerable resilience to various abiotic stresses (Jamil et al. 2018; Oguis et al. 2019). Given its broad utility and ecological adaptability, the underutilization of this species underscores the need for enhanced cultivation, research, and conservation efforts, particularly in biodiversity-rich countries like Indonesia (Afrianto and Metananda 2023; Filio et al. 2023). In order to support its utilization and conservation, assessing the genetic diversity of *C. ternatea* using molecular markers, such as SRAP, is necessary.

One commonly used method for genetic diversity analysis is the SRAP (Sequence-Related Amplified Polymorphism) marker (Nabilla et al. 2021). To gain deeper insights into genotype-phenotype relationships, SRAP markers can be effectively employed to identify associations between molecular markers and phenotypic traits of interest (Khaled et al. 2019). SRAP markers were employed due to their simplicity, high reproducibility, and ability to target open reading frames, which are often associated with agronomic traits (Alghamdi et al. 2019). SRAP markers have been reported as effective tools for identifying genetic diversity in both cultivated and wild plant species. For example, the Fabaceae family has been successfully applied to *Vigna radiata* genotypes (Fatmawati et al. 2023) and wild species such as *Dalbergia latifolia* (Yulita et al. 2020). SRAP markers successfully confirmed the distinct taxonomic status of *Echinosophora koreensis* (now *Sophora koreensis*) within the Fabaceae family (Ho et al. 2024). These markers have demonstrated high effectiveness in detecting polymorphism and differentiating genotypes, as shown in genetic diversity and structure of *Quercus petraea* (Rebrean et al. 2023). In comparison to ISSR markers, SRAP markers demonstrated a greater effective multiplex ratio in terms of their polymorphism information content (Hancı and Paşazade 2025). However, SRAP markers can not distinguish between homozygous and heterozygous genotypes, which limits their utility in fine-scale population genetic analyses (Yan et al. 2019).

Genetic studies on *C. ternatea* are still relatively limited compared to other plant species. Previous ISSR analysis research conducted in North Maluku, Indonesia, found significant genetic diversity among 18 accessions (Nurhasanah et al. 2023). In contrast, studies conducted in Yogyakarta demonstrated that the Internal Transcribed Spacer (ITS) region was highly conserved and thus insufficient to differentiate among genotypes (Yusuf et al. 2025). The integration of morphological traits with Start Codon Targeted (SCoT) molecular markers has successfully distinguished seven distinct morphotypes of *C. ternatea*, primarily based on variations in floral characteristics (Arya et al. 2024). Expanding the molecular applications above, the study can be used to identify genetic characterization using SRAP markers, which has introduced a novel approach focusing on flower colour variations and petal morphology. This study aimed to assess genetic diversity among eight *C. ternatea* accessions with varying flower colors and petal types, and evaluate SRAP marker efficiency.

MATERIALS AND METHODS

Plant materials

The research materials were eight accessions of *Clitoria ternatea* that had different flower color characteristics and numbers of petals (Table 1). The accessions used included 1U (purple flower-single petal), 2U (purple flower-double petal), 1B (blue flower-single petal), 2B (blue flower-double petal), 1Pn (pink flower-single petal), 2Pn (pink flower-double petal), 1Pt (white flower-single petal), and 2Pt (white flower-double petal) (Table 1). All eight accessions were obtained from a local nursery in Malang, East Java, Indonesia.

Procedures

DNA isolation

Approximately 100 mg of fresh leaf samples were thoroughly washed under running water and dried using tissue paper. Total DNA was isolated using the CTAB (Cetyltrimethylammonium Bromide) method described by Doyle (1991). The leaf samples were finely ground using a mortar and pestle, followed by the addition of 1,500 µL of CTAB buffer solution containing 2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl pH 8, 20 mM EDTA pH 8, 2% PVP, and 2% β-mercaptoethanol, which had been pre-incubated in a water bath at 65°C for 30 minutes. The resulting mixture was then incubated at 65°C for 60 minutes, with occasional mixing every 10 minutes to ensure homogeneity.

After the 60-minutes incubation, the mixture was removed from the water bath and allowed to stand for 2 minutes. Subsequently, 500 µL of a 24:1 chloroform: isoamyl alcohol (CIAA) mixture was added to each sample, vortexed for 5 minutes, and centrifuged at 12,000 rpm for 15 minutes. The resulting supernatant was carefully collected and mixed with sodium acetate at a volume equal to 1/10 of the supernatant volume. Cold isopropanol was added, equivalent to 2/3 of the total volume (supernatant + sodium acetate). The mixture was gently inverted to ensure proper mixing and incubated at 4°C for 1 to 24 hours, followed by centrifugation at 12,000 rpm for 10 minutes. The supernatant was discarded, and the DNA pellet was washed with 500 µL of 70% ethanol, followed by centrifugation at 12,000 rpm for 5 minutes. The DNA pellet was then subjected to a second wash using 500 µL of absolute ethanol, followed by centrifugation at 12,000 rpm for 5 minutes. The supernatant was discarded, and the DNA pellet was dried. Once dried, the DNA pellet was dissolved in 50 µL of double-distilled water (ddH_2O, Aquabides) and stored in a refrigerator at 4°C.

Table 1. Flower and leaf variation of *Clitoria ternatea* accessions used in the study

Accession	Characteristic		
	Flower	Leaf	Figure
1U	Purple	Single	
2U	Purple	Double	
1B	Blue	Single	
2B	Blue	Double	
1Pn	Pink	Single	
2Pn	Pink	Double	
1Pt	White	Single	
2Pt	White	Double	

Table 2. List of primers used in DNA amplification

Forward	Sequence 5'-3'	Reverse	Sequence 5'-3'	Primer combination
Me3	TGA GTC CAA ACC GGA AT	Em3	GAC TGC GTA CGA ATT GAC	Me3Em3
Me3	TGA GTC CAA ACC GGA AT	Em4	GAC TGC GTA CGA ATT TGA	Me3Em4
Me2	TGA GTC CAA ACC GGA GC	Em4	GAC TGC GTA CGA ATT TGA	Me2Em4
Me3	TGA GTC CAA ACC GGA AT	Em2	GAC TGC GTA GCA ATT TGC	Me3Em2
Me2	TGA GTC CAA ACC GGA GC	Em1	GAC TGC GTA CGA ATT AAT	Me2Em1
Me2	TGA GTC CAA ACC GGA GC	Em2	GAC TGC GTA GCA ATT TGC	Me2Em2
Me2	TGA GTC CAA ACC GGA GC	Em3	GAC TGC GTA CGA ATT GAC	Me2Em3
Me1	TGA GTC CAA ACC GGA TA	Em2	GAC TGC GTA GCA ATT TGC	Me1Em2
Me4	TGA GTC CAA ACC GGA CC	Em3	GAC TGC GTA CGA ATT GAC	Me4Em3
Me4	TGA GTC CAA ACC GGA CC	Em4	GAC TGC GTA CGA ATT TGA	Me4Em4

The purified DNA was quantified using a Gene Quant Spectrophotometer to determine the DNA concentration obtained. The concentration of the DNA sample was measured based on light absorption at 260 nm. DNA dilution was performed to obtain the appropriate DNA concentration for amplification. Next, to achieve the desired final dilution volume, the DNA solution was diluted with ddH_2O to adjust the DNA concentration to the required level for PCR. The dilution was calculated using the formula described by Nandiyanto et al. (2023) as follows:

$$V_1 . M_1 = V_2 . M_2$$

Where :
M_1 : DNA sample concentration
V_1 : Initial volume of the sample to be diluted
M_2 : 100 ng/µL
V_2 : Final volume
Dilution volume : $V_2 - V_1$

DNA amplification

DNA amplification was performed using the Polymerase Chain Reaction (PCR) technique to replicate DNA sequences using specific primers. The PCR conditions followed the protocol established by Li and Quiros (2001). The list of primers used in DNA amplification is presented in Table 2 (Nabilla et al. 2021). The PCR was conducted in a total volume of 12.5 µL for each PCR tube. The reaction mixture for PCR with SRAP markers consisted of 6.25 µL of master mix (PowerPol), 3.25 µL of nuclease-free water, 1 µL of SRAP primer, and 2 µL of DNA template with a concentration of 100 ng/µL. Amplification was performed in a BIO-RAD T100 thermal cycler using several steps as follows: an initial denaturation at 94°C for 5 minutes to denature the DNA strands. This was followed by 5 pre-cycles consisting of denaturation at 94°C for 1 minute, annealing at 35°C for 1 minute, and extension at 72°C for 1 minute. Subsequently, 35 amplification cycles were performed with the same thermal profile: denaturation at 94°C for 1 minute, annealing at 35°C for 1 minute, and extension at 72°C for 1 minute. The reaction was completed with a final extension step at 72°C for 8 minutes to ensure complete synthesis of the PCR products.

The amplified DNA was then subjected to electrophoresis using a horizontal electrophoresis tank. A 1.5% (b/v) agarose gel was prepared by dissolving agarose powder in 1X TBE buffer, which consists of 0.45 M Tris-HCl (pH 8), 0.45 M boric acid, and 20 mM EDTA. FloroSafe DNA was added to the gel to enable visualization of the DNA bands. Electrophoresis was carried out at 100 V and a current of 400 mA for 75 minutes. The results were then visualized using the UV-Vis spectrophotometry NanoDrop 2000/c (Thermo Fisher Scientific).

Data analysis

The gel electrophoresis results were scored using binary data based on the presence or absence of DNA bands, where the presence of a band was assigned a value of "1" and the absence of a band was assigned a value of "0". To ensure data reliability, all PCR amplifications were performed in duplicate for each primer combination. Only clear and reproducible bands that appeared consistently in both independent runs were considered for scoring and subsequent data analysis. The parameters assessed included the Number of Polymorphic Bands (NPB), the Percentage of Polymorphism (PP), Polymorphic Information Content (PIC), Resolving Power (RP), and Marker Index (MI). NTB was calculated by counting the number of clear DNA bands generated through PCR amplification. NPB was determined based on the distinct positions of the DNA bands across the lanes. PP was calculated as the ratio of NPB to NTB. Botstein et al. (1980) stated that the PIC value can be calculated using the following equation:

$$PIC = 1 - \sum_{j=1}^{n} Pij^2$$

Where :
PIC : Polymorphic Information Content
n : Number of alleles or bands observed at a given locus
Pij : Frequency of the jth allele or band for the ith marker

The MI was calculated by the PIC × the number of polymorphic bands (Powell et al. 1996). RP is calculated by summing the informativeness of each band using the formula RP = Σ Ib, where Ib is determined by the equation $1 - (2 \times |0.5 - p|)$, with p representing the proportion of genotypes in which the band is present (Rini et al. 2023). The binary data obtained were further analyzed using GenAlex 6.5 software, a powerful tool in genetic analysis, to generate a Principal Coordinate Analysis (PCoA) plot, visually representing the samples' genetic relationships. For cluster analysis, the data were processed using NTSYS 2.02 software to construct a dendrogram representing the genetic relatedness of the samples. The cluster analysis was

visualized using the Unweighted Pair Group Method with Arithmetic mean (UPGMA). The Dice similarity coefficient values were used to determine the degree of genetic relatedness between samples, providing insights into their genetic diversity and potential clustering patterns. The cophenetic correlation coefficient was calculated by measuring the Pearson correlation between the original pairwise genetic distance matrix and the cophenetic distance matrix obtained from the dendrogram as follows:

$$r = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2} \times \sqrt{\sum(y_i - \bar{y})^2}}$$

Where:

r : Cophenetic Correlation Coefficient

x_i : Original genetic distance between sample pair i

y_i : Cophenetic distance between the same sample pair i

$\bar{x}$: mean of all original distance values

$\bar{y}$: mean of all cophenetic distance values

$\sum$: Summation over all pairs of samples

The population structure was examined using a Bayesian model-based clustering approach implemented in STRUCTURE version 2.3.4 (Pritchard et al. 2000) to identify the number of distinct genetic clusters (K) and analyze the genetic composition of the sampled populations. The analysis involved a burn-in phase of 20,000 steps, followed by 100,000 steps of Markov Chain Monte Carlo simulation, conducted with 10 independent runs for each K value (K = 1 to 10). The Evanno method was applied to identify the optimal K value, and population structure visualization was conducted using PopHelper (Francis 2016).

RESULTS AND DISCUSSION

DNA quality and quantity

The DNA quantification results obtained using the Gene Quant Spectrophotometer demonstrated considerable variation in DNA concentration and purity among the samples, showing significant variation in concentration and purity across the samples (Table 3). The highest DNA concentration was recorded in sample 1U (9500 ng/μL), while the lowest was found in sample 2B (2900 ng/μL). The purity values, measured through the A260/A280 ratio, ranged from 1.36 to 1.86. Most samples exhibited ratios close to 1.80, which indicates minimal contamination by proteins or other impurities. On the other hand, sample 1B, which showed a relatively low purity value (1.36), suggests the presence of contaminants that could affect downstream applications such as PCR.

DNA polymorphism

The primer combinations used in this study include several combinations, such as Me3/Em3, Me3/Em4, Me2/Em4, Me3/Em2, Me2/Em1, Me2/Em2, Me2/Em3, Me1/Em2, Me4/Em3, and Me4/Em4 (Figure 1). The resulting band patterns showed variation among accessions studied. Differences in band intensity and number reflected the degree of genetic polymorphism among the samples, thereby supporting the genetic diversity analysis. The clear band patterns can be interpreted to differentiate the samples. Similar band patterns across samples indicate conserved DNA regions, while unique bands reflect specific genetic variations.

Table 3. DNA purity and concentration value results

Sample code	Purity (A260/A280)	Concentration (ng/μL)
1U	1.86	9500
1B	1.36	3400
1Pn	1.85	7200
1Pt	1.60	7500
2U	1.61	6300
2B	1.81	2900
2Pn	1.85	6100
2Pt	1.86	5400

The analysis of various primer combinations in this molecular study reveals significant genetic diversity across different samples (Table 4). The size range of amplified DNA fragments varied from 150 to 1500 bp from a total NTB of 114 bands. Among these, two bands exhibited PP, achieved 100% polymorphism, such as Me3Em3 and Me4Em4, achieving 100% polymorphism. The total NPB was 101 polymorphic, with an average of 10 per primer. The PIC, which measures the informativeness of markers, ranged from 0.25 to 0.37, with an overall average of 0.31. These values indicate that the primers used were moderately informative for genetic analysis. The RP ranged from 3.00 to 10.50, with an overall average of 5.50, and the MI ranged from 1.27 to 6.51, with an overall average of 3.24. These values showed the markers' ability to differentiate the accessions and their usefulness in capturing genetic diversity across the samples.

Genetic similarity and clustering

The genetic similarity coefficient of Dice for *C. ternatea* accessions analyzed using SRAP markers is presented in Table 5. The similarity values reflect varying degrees of genetic relatedness among the accessions, with higher coefficients indicating closer genetic relationships and lower values representing greater divergence. The highest similarity value observed among different samples was 0.75 between samples 1Pt and 2B, suggesting a high degree of genetic relatedness between them. Conversely, the lowest similarity value of 0.42 was observed between samples 1U and 1B, indicating a relatively lower genetic resemblance.

The cophenetic correlation coefficient for the given data is approximately 0.79. This indicates a moderate level of agreement between the pairwise distances of the original data and those obtained from the hierarchical clustering. A dendrogram illustrating the clustering of *C. ternatea* samples based on SRAP markers is presented in Figure 2.A. The hierarchical clustering method in the dendrogram groups the samples according to their genetic similarity, with closer branches indicating higher genetic relatedness. Clustered samples share a higher degree of genetic similarity, while those positioned further apart exhibit greater genetic divergence. The dendrogram reveals that there were two clusters: cluster 1 (1U, 1Pn, 2U, and 2Pn) and cluster 2 (1B, 1Pt, 2B, and 2Pt). Additionally, the results of the PCoA (Figure 2.B) were aligned with the dendrogram. Accessions grouped in the dendrogram were also

positioned closely in the PCoA plot, confirming the genetic differentiation observed across the two analytical methods.

Table 4. Genetic diversity of *Clitoria ternatea* accession by SRAP primers

Primer	Size range (bp)	NTB	NPB	PP (%)	PIC	RP	MI
Me1Em2	150-750	6.00	5.00	83.33	0.32	3.00	1.61
Me2EM1	120-1030	13.00	11.00	84.62	0.25	4.25	2.78
Me2Em2	180-1000	12.00	10.00	83.33	0.31	6.00	3.07
Me2Em3	200-1000	7.00	6.00	85.71	0.32	3.50	1.93
Me2Em4	180-1350	8.00	5.00	62.50	0.25	3.25	1.27
Me3Em2	150-1500	13.00	11.00	84.62	0.30	6.00	3.28
Me3Em3	150-800	12.00	12.00	100.00	0.34	5.75	4.03
Me3Em4	150-1350	13.00	12.00	92.31	0.37	7.50	4.38
Me4Em3	160-1300	19.00	18.00	94.74	0.36	10.50	6.51
Me4Em4	200-1100	11.00	11.00	100.00	0.32	5.25	3.53
Total	-	114.00	101.00	-	-	-	-
Mean	-	11.40	10.10	87.12	0.31	5.50	3.24

Note: NTB: Number of Total Bands, NPB: Number of Polymorphic Bands, PP: Percentage of Polymorphism, PIC: Polymorphic Information Content, RP: Resolving Power, and MI: Marker Index

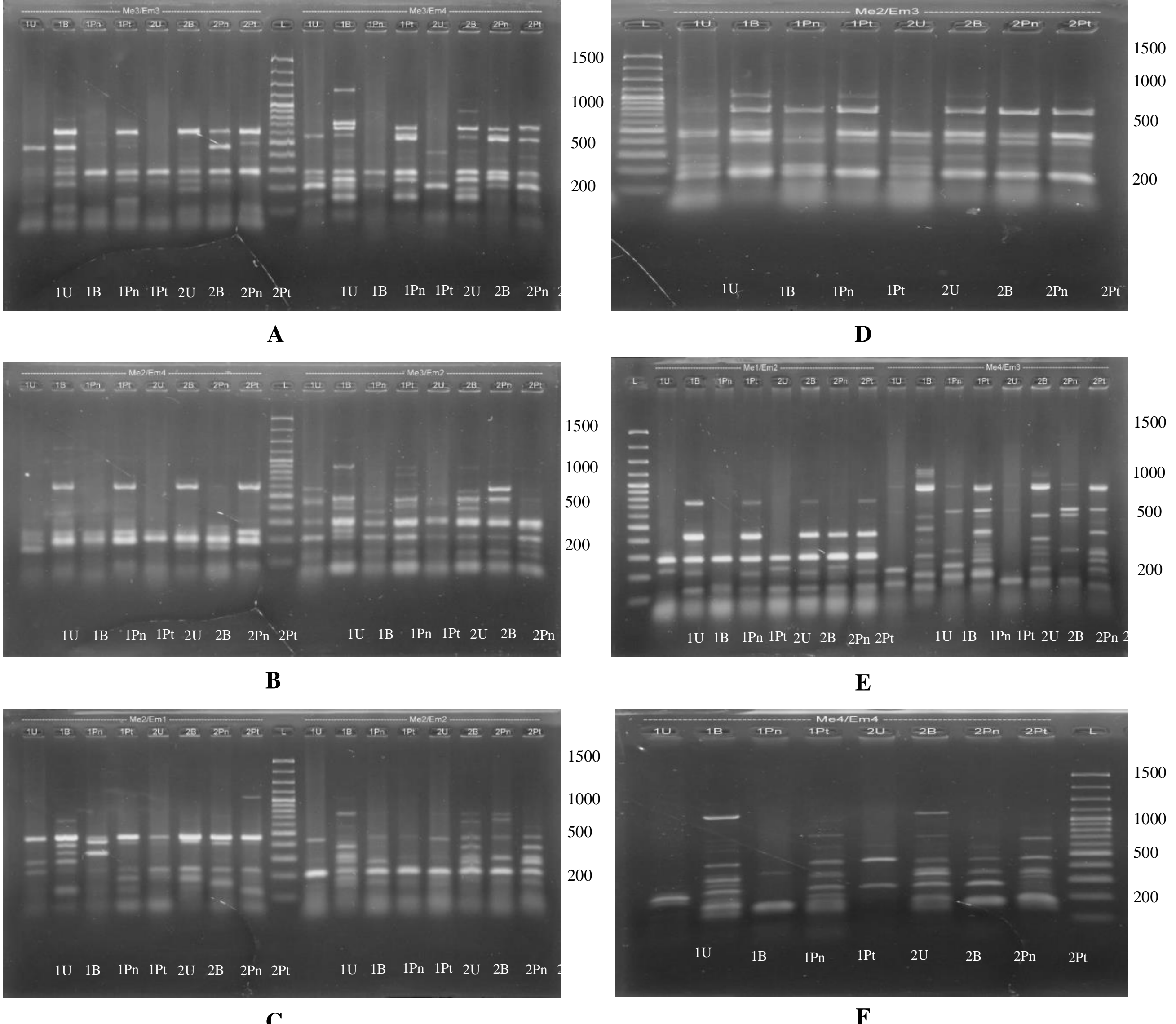

Figure 2. Banding patterns of *Clitoria ternatea* generated by SRAP markers: A. Me3/Em3; Me3/Em4, B. Me2/Em4; Me3/Em2, C. Me2/Em1; Me2/Em2, D. Me2/Em3, E. Me1/Em2; Me4/Em3, and F. Me4/Em4

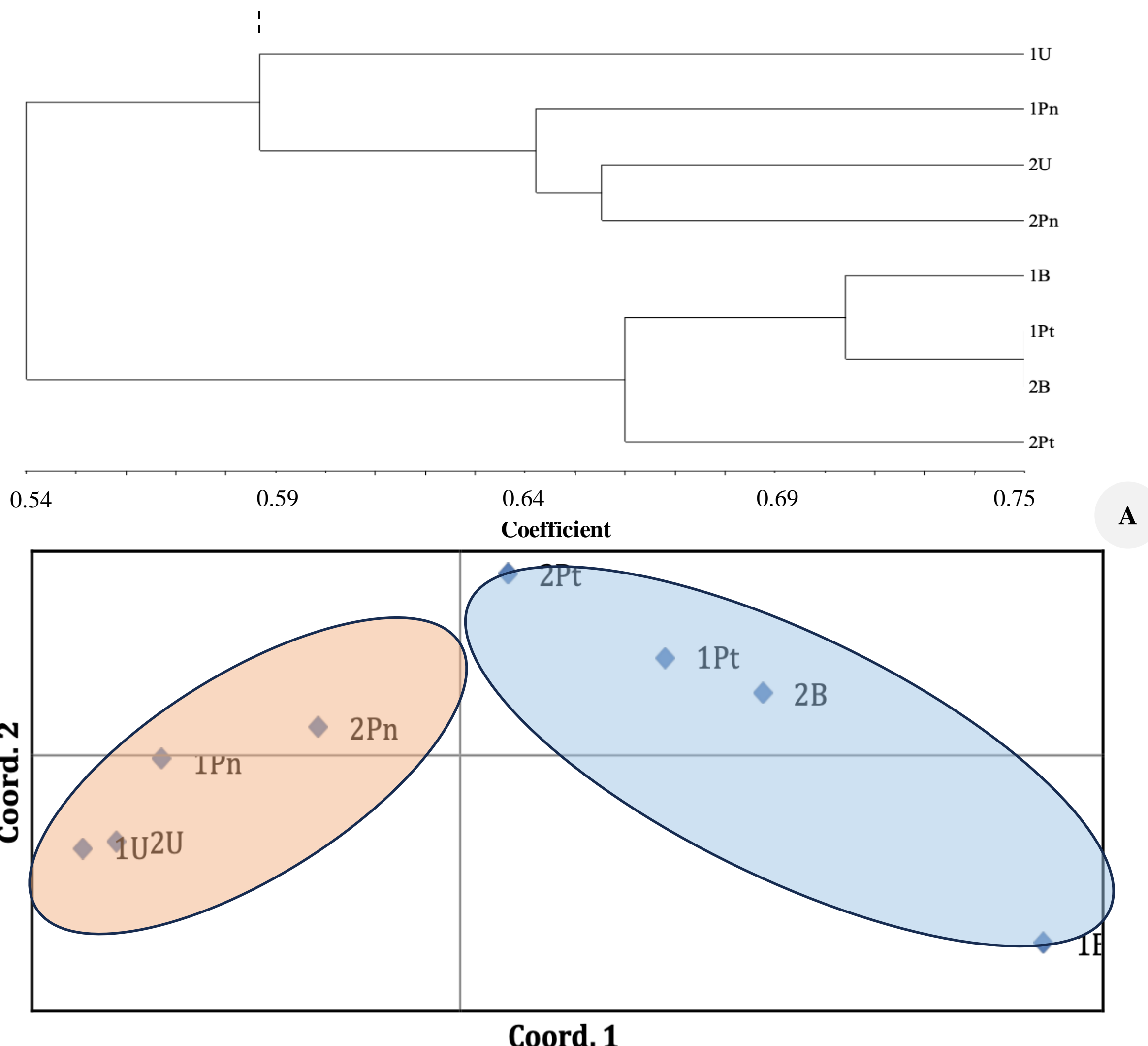

Figure 2. Clustering analysis of eight accessions of *Clitoria ternatea* using SRAP markers: A. Dendrogram, and B. Biplot [illegible]ncipal Coordinate Analysis (PCoA)

Table 5. Genetic similarity coefficient of dice in *Clitoria ternatea* samples using SRAP markers

	1U	1B	1Pn	1Pt	2U	2B	2Pn	2Pt
1U	1.00							
1B	0.42	1.00						
1Pn	0.55	0.48	1.00					
1Pt	0.47	0.69	0.57	1.00				
2U	0.60	0.45	0.65	0.53	1.00			
2B	0.48	0.73	0.53	0.75	0.52	1.00		
2Pn	0.61	0.58	0.64	0.70	0.66	0.69	1.00	
2Pt	0.46	0.60	0.62	0.70	0.46	0.69	0.65	1.00

The population structure of the eight *C. ternatea* accessions was further analyzed using STRUCTURE software based on Bayesian clustering. The optimal number of genetic clusters (K) was estimated by applying the Evanno method, which evaluates the rate of change in the log-likelihood of data between successive K values. The mean log-likelihood values [LnP(K)] increased with higher K values but showed greater variance beyond K = 2 (Figure 3.A). The corresponding ΔK plot in Figure 3.B revealed a distinct peak at K = 2, indicating that two genetic clusters best represent the underlying population structure.

The population structure of the eight *C. ternatea* accessions based on the optimal K value (ΔK = 2) determined by the Evanno method is presented in Figure 4. Each individual was represented by a vertical bar divided into two colored segments, with blue representing Cluster 1 and yellow representing Cluster 2. The x-axis showed the accession codes, while the y-axis indicated the membership probability for each genetic cluster. The first four accessions (1U, 1B, 1Pn, and 1Pt) exhibited higher membership proportions in Cluster 1, suggesting genetic similarity within this group. In contrast, the remaining four accessions (2U, 2B, 2Pn, and 2Pt) predominantly belonged to Cluster 2. This clear separation reflects distinct genetic structuring

among the accessions, which may correspond to differences in phenotypic traits such as petal type and flower color.

Discussion

The purity is considered acceptable within the range of 1.80-2.00 to ensure high-quality amplification (Russo et al. 2022). The restricted DNA formed a smear on a 1% agarose gel, suggesting complete digestion of the DNA samples and confirming their purity (Irsyadi et al. 2024). Differences in DNA quality, such as the low purity observed in sample 1B (A260/A280 = 1.36), may have negatively impacted PCR amplification efficiency and band clarity. Contaminants such as proteins or phenol, not completely removed during extraction, may inhibit enzymatic activity during PCR. Overall, the purity result was lower than the study by Nabilla et al. (2021) on *Piper betle*, which reported values of 1.73-2.39. Meanwhile, Delfianti et al. (2021) on *Diospyros kaki* also showed higher values of 1.91-1.97.

The SRAP markers proved effective in detecting genetic variation among *C. ternatea* accessions. With a high percentage of polymorphic bands, these markers demonstrated sufficient resolution to discriminate between genetically distinct accessions. The informative value of SRAP lies in its tendency to target coding regions, making it more reproducible than RAPD and ISSR, although often slightly less polymorphic (Yulita et al. 2020; Rini et al. 2023). Moreover, the values obtained in this study surpass those reported by Wardi et al. (2024), where the PP was below 60% in the SRAP analysis of *Uncaria gambir*. It was also higher than those reported by Erlinawati et al. (2018), which showed an average PP of 85.03% in the SRAP analysis of *Cyrtosperma merkusii*. Rini et al. (2023) showed the PIC value was lower (0.2816), while the RP and MI values were notably higher, at 9.13 and 3.80, respectively, on Indonesian local rice genotypes (*Oryza sativa*).

A dendrogram generated by UPGMA and a PCoA plot revealed two consistent clusters aligned with petal color and morphology. Cluster I (1U, 1Pn, 2U, 2Pn) predominantly included pink and purple pigmentation accessions, while Cluster II (1B, 2B, 1Pt, 2Pt) contained blue and white types. This finding aligns with earlier studies that linked flower morphology with genetic profiles. For instance, Arya et al. (2024) demonstrated that petal shape and pigmentation of *C. ternatea* were critical in separating morphotypes using SCoT markers. Similarly, Bhusan (2024) found specific combinations of petal number and color of *C. ternatea* associated with closer genetic distances using RAPD. However, differing from Kholifah (2024), *rbcL*-based analysis showed no genetic differences among *C. ternatea* petal and color variants, with all samples forming one clade (99% bootstrap, 0.00% distance).

Pigmentation differences in butterfly pea are largely attributed to variations in anthocyanin content, which is known to be genetically regulated. Handayani et al. (2024) demonstrated that different extraction and environmental conditions of *C. ternatea* can affect anthocyanin profiles, but these differences are still rooted in genetic potential. The variation in petal color of *C. ternatea* has been shown to strongly correlate with differences in anthocyanin content (Maulani 2022), a relationship that enlightens our understanding of plant biochemistry. Furthermore, according to Maulani (2022), flowers with double petals and purple coloration exhibited notably higher anthocyanin levels. According to Havananda and Luengwilai (2019), blue petals of *C. ternatea* had significantly higher anthocyanin concentrations compared to lighter-colored petals, such as mauve or white, which possess markedly lower or negligible anthocyanin content. According to Lakshan et al. (2020), the chemical properties of *C. ternatea* varied not only with petal color but also with keel type (normal or enlarged). The pigmentation intensity and floral chromatic attributes of *C. ternatea* play a key role in determining the biochemical composition of the flowers (Surya et al. 2023).

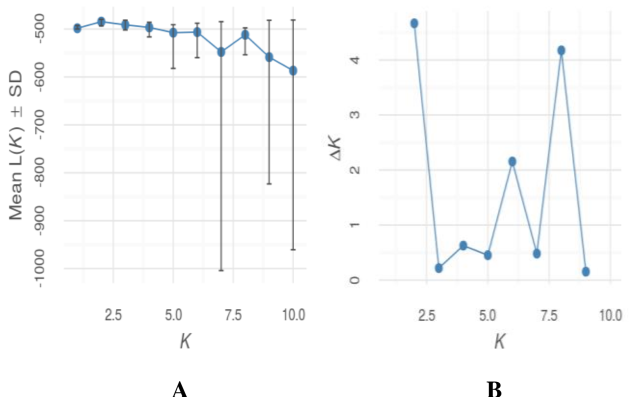

Figure 3. Evaluating the population structure of *Clitoria ternatea*: A. Mean log likelihood [L(K)] ± SD for each K, B. ΔK values from the Evanno method to determine optimal K

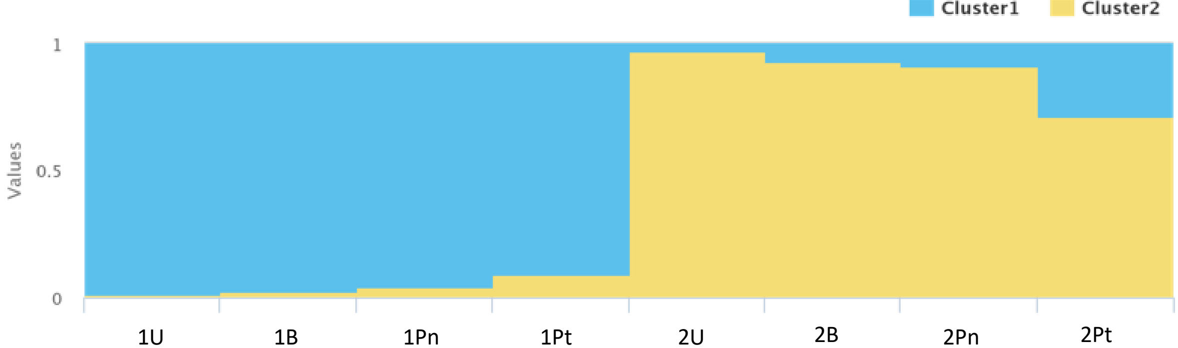

Figure 4. Population structure of eight *Clitoria ternatea* accessions at K = 2 based on STRUCTURE analysis. Each bar represents an individual accession, with colors showing membership proportions in two genetic clusters (blue for Cluster 1, yellow for Cluster 2). The x-axis shows accession codes, and the y-axis represents membership coefficients (Q values)

Environmental heterogeneity and reproductive biology shape genetic patterns in *C. ternatea*. Populations exposed to diverse environmental pressures tend to maintain higher genetic diversity (Stojnić et al. 2019; Saswita et al. 2023). This diversity may also arise from varying levels of environmental selection or multiple introduction sources (Nurhasanah et al. 2023). Phenotypic differences observed among *C. ternatea* accessions from various regions of Indonesia and Thailand further support this, as these variations are driven by local environmental factors (Aziza et al. 2021). Moreover, *C. ternatea* showed strong adaptability and variable phytochemical expression under different environmental settings (Jamil et al. 2018). Environmental factors and genotype-environment interactions significantly influenced the yield and related traits of this species (Filio et al. 2023).

In this study, the optimal number of clusters was determined as K=2. The analysis indicated partial population divergence and gene flow (Yulita et al. 2022). These clusters may reflect domestication events or human-driven selection for floral traits, a pattern also reported in other plant species. For instance, *Tectona grandis* exhibited two distinct clusters linked to domestication and selective breeding (Nurtjahjaningsih et al. 2023), while *Psophocarpus tetragonolobus* also showed K = 2 clustering patterns influenced by domestication history and geographical differentiation (Kumar and Rajalakshmi 2023). Similarly, *Astragalus exscapus* subsp. *transsilvanicus* displayed clear K = 2 clustering that corresponded with population structure (Szabo et al. 2021). SRAP-based STRUCTURE analyses in other species, such as *Mallotus oblongifolius* and *Saussurea medusa*, confirmed K = 2 as the optimal grouping, highlighting the roles of natural adaptation, selective pressures, and geographical isolation (Yan et al. 2019; Wang et al. 2023). Likewise, *Cucumis melo* from the Kurdistan region of Iraq exhibited K = 2, demonstrating clear genetic subdivision among melon genotypes consistent with population differentiation (Aziz and Tahir 2023).

In conclusion, this study successfully analyzed the genetic diversity of eight accessions of *C. ternatea* using SRAP markers. A total of 114 DNA bands were amplified, of which 101 were polymorphic, with an average PP of 87.12%, indicating a high level of genetic variability. PIC averaged 0.31, while RP and MI averaged 5.5 and 3.25, respectively. The results of cluster analysis (UPGMA), PCoA, and population structure analysis using STRUCTURE software consistently identified two main genetic clusters corresponding to differences in petal type and flower color. Based on this study, the genetic differences provide several practical applications for plant breeding, conservation, and germplasm classification. For plant breeders, accessions from different clusters can be crossed to create new varieties with more attractive flower shapes or colors, or with higher levels of beneficial compounds for health and nutrition. Regarding conservation, these findings help identify which genetically unique accessions are worth prioritizing, either to be protected in their natural habitats (in situ) or stored safely in seed banks (ex situ). Additionally, this helps in organizing and classifying butterfly pea collections more efficiently and meaningfully for breeding programs, research, and bioprospecting efforts to discover new uses for this versatile plant.

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REFERENCES

Afrianto WF, Tamnge F, Hasanah LN. 2020. A relation between ethnobotany and bioprospecting of edible flower butterfly pea (*Clitoria ternatea*) in Indonesia. Asian J Ethnobiol 3 (2): 51-61. DOI: 10.13057/asianjethnobiol/y030202.

Afrianto WF, Tamnge F, Hidayatullah T, Hasanah LN. 2021. Local knowledge of plant-based nutrition sources from forgotten foods in Datengan Village, East Java, Indonesia. Asian J Ethnobiol 4 (1): 53-64. DOI: 10.13057/asianjethnobiol/y040106.

Afrianto WF, Metananda AA. 2023. Revealing the biocultural importance of *Moringa oleifera* (Moringaceae) in three villages, Kediri District, Indonesia. Biodiversitas 24: 6942-6952. DOI: 10.13057/biodiv/d241256.

Alghamdi SS, Khan MA, Migdadi HM, El-Harty EH, Afzal M, Farooq M. 2019. Biochemical and molecular characterization of cowpea landraces using seed storage proteins and SRAP marker patterns. Saudi J Biol Sci 26 (1): 74-82. DOI: 10.1016/j.sjbs.2018.09.004.

Arya MR, Beevy SS, Dan M. 2024. Morphological and genetic diversity of Indian *Clitoria ternatea* L. (Faboideae, Fabaceae) morphotypes inferred from floral characters and ScoT markers. Plant Mol Biol Rep 43: 815-832. DOI: 10.1007/s11105-024-01509-7.

Aziz RR, Tahir NA-R. 2023. Genetic diversity and structure analysis of melon (*Cucumis melo* L.) genotypes using URP, SRAP, and CDDP markers. Genet Resour Crop Evol 70: 799-813. DOI: 10.1007/s10722-022-01462-y.

Aziza V, Ulimaz TA, Ustari D, Suganda T, Concibido V, Irawan B, Karuniawan A. 2021. Phenotypic diversity of double petal butterfly pea from Indonesia and Thailand based on flower morphology. Al-Kauniyah J Biol 14 (1): 78-89. DOI: 10.15408/kauniyah.v14i1.15558. [Indonesian]

Bhusan A. 2024. Morphological, biochemical and molecular diversity analysis in *Clitoria ternatea* L. [Dissertation]. Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India.

Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32 (3): 314-331.

Delfianti MNI, Yuniastuti E, Cahyani VR. 2021. AFLP primer selection for the analysis of genetic diversity in persimmon (*Diospyros kaki* L.) originated from Central and East Java, Indonesia. Jordan J Biol Sci 14 (3): 471-475. DOI: 10.54319/jjbs/140312.

Doyle J. 1991. DNA protocols for plants. In: Hewitt GM, Johnston AWB, Young JPW (eds). Molecular Techniques in Taxonomy. NATO ASI Series Vol 57. Springer, Berlin, Heidelberg. DOI: 10.1007/978-3-642-83962-7_18.

Erlinawati I, Abinawanto A, Salamah A, Rugayah R. 2018. Genetic diversity analysis of daluga (*Cyrtosperma merkusii*) using sequence-related amplified polymorphism in Siau, Sangihe and Talaud Islands, North Sulawesi, Indonesia. Biodiversitas 19 (6): 2374-2380. DOI: 10.13057/biodiv/d190648.

Fatmawati Y, Ilyas I, Setiawan AB, Purwantoro A, Respatie DW, Teo CH. 2023. Genetic evaluation of F2 and F3 interspecific hybrids of mung bean (*Vigna radiata* L. Wilczek) using retrotransposon-based insertion polymorphism and sequence-related amplified polymorphism

markers. Indones J Biotechnol 28 (3): 143-152. DOI: 10.22146/ijbiotech.82760.

Filio YL, Maulana H, Aulia R, Suganda T, Ulimaz TA, Aziza V, Concibido V, Karuniawan A. 2023. Evaluation of Indonesian butterfly pea (*Clitoria ternatea* L.) using stability analysis and sustainability index. Sustainability 15 (3): 2459. DOI: 10.3390/su15032459.

Francis RM. 2016. POPHELPER: An R package and web app to analyse and visualise population structure. Mol Ecol Resour 17 (1): 27-32. DOI: 10.1111/1755-0998.12509.

Hancı F, Paşazade E. 2025. A Comparison of efficiency parameters of SRAP and ISSR markers in revealing variation in *Allium* germplasm. Horticulturae 11 (3): 294. DOI: 10.3390/horticulturae11030294.

Handayani L, Aprilia S, Arahman N, Bilad MR. 2024. Identification of the anthocyanin profile from butterfly pea (*Clitoria ternatea* L.) flowers under varying extraction conditions: Evaluating its potential as a natural blue food colorant and its application as a colorimetric indicator. S Afr J Chem Eng 49: 151-161. DOI: 10.1016/j.sajce.2024.04.008.

Havananda T, Luengwilai K. 2019. Variation in floral antioxidant activities and phytochemical properties among butterfly pea (*Clitoria ternatea* L.) germplasm. Genet Resour Crop Evol 66: 645-658. DOI: 10.1007/s10722-018-00738-6.

Ho U-H, Pak S-H, Song S-R, Won H-M, Ju I-Y, Ri S-J, Ho T-S, Ri J-K, Pak M-H. 2024. Phylogenetic study on some species of Fabaceae using morphological traits and Sequence Related Amplified Polymorphism (SRAP) markers. Genet Resour Crop Evol 71: 4929-4939. DOI: 10.1007/s10722-024-01936-1.

Irsyadi MB, Sari SK, Oktiastuti E, Rineksane IA. 2024. Rapid genomic DNA extraction for Soybean (*Glycine max* L. Merr) using modified CTAB protocol to obtain high-quality DNA. Indian J Biochem Biophys 61 (2): 97-104. DOI: 10.56042/ijbb.v61i2.7603.

Jamil N, Zairi MNM, Nasim NAM, Pa'ee F. 2018. Influences of environmental conditions to phytoconstituents in *Clitoria ternatea* (butterfly pea flower)-A review. J Sci Technol 10 (2): 208-228. DOI: 10.30880/jst.2018.10.02.029.

Khaled AGA, El-Sherbeny GAR, Elsayed HMA. 2019. Three SRAP molecular markers linked to yield component traits in wheat. Asian J Res Rev Agric 1 (1): 25-35.

Kholifah AN. 2024. Phylogenetic analysis of *Clitoria ternatea* (butterfly pea) based on *rbcL* gene (large subunit ribulose 1,5 biphosphate carboxylase/oxygenase). [Thesis]. UIN Syarief Hidayatullah, Jakarta. [Indonesian]

Kumar VK, Rajalakshmi R. 2023. Population diversity analysis of an underutilized legume, winged bean (*Psophocarpus tetragonolobus* (L.) DC.) using ISSR markers. Plant Gene 36: 100436. DOI: 10.1016/j.plgene.2023.100436.

Lakshan SAT, Pathirana CK, Jayanath NY, Abeysekara WPKM, Abeysekara WKSM. 2020. Antioxidant and selected chemical properties of the flowers of three different varieties of butterfly pea (*Clitoria ternatea* L.). Ceylon J Sci 49 (2): 195-201. DOI: 10.4038/cjs.v49i2.7740.

Li G, Quiros CF. 2001. Sequence-Related Amplified Polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in *Brassica*. Theor Appl Genet 103: 455-461. DOI: 10.1007/s001220100570.

Maulani AA. 2022. Morphological characterization and anthocyanin content of butterfly pea (*Clitoria ternatea* L.) flowers with different colors and petal numbers. [Dissertation]. Universitas Gadjah Mada, Yogyakarta. [Indonesian]

Nabilla S, Safira UM, Puspita PJ, Subositi D, Maruzy A, Artika IM. 2021. Genetic diversity analysis of *Piper betle* from eight accessions of Indonesia based on SRAP markers. Biodiversitas 22 (8): 3401-3408. DOI: 10.13057/biodiv/d220837.

Nandiyanto ABD, Ragadhita R, Aziz M. 2023. How to calculate and measure solution concentration using UV-Vis spectrum analysis: Supporting measurement in the chemical decomposition, photocatalysis, phytoremediation, and adsorption process. Indones J Sci Technol 8 (2): 345-362. DOI: 10.17509/ijost.v8i2.57783.

Nurhasanah, Hindersah R, Suganda T, Concibido V, Sundari, Karuniawan A. 2023. The first report on the application of ISSR markers in genetic variance detection among butterfly pea (*Clitoria ternatea* L.) accession in North Maluku Province, Indonesia. Horticulturae 9 (9): 1059. DOI: 10.3390/horticulturae9091059.

Nurtjahjaningsih ILG, Rimbawanto A, Fauzi MA, Dormontt EE, Lowe AJ, Hendrati RL, Baskorowati L, Susanto M, Sulistiadi HBS, Mashudi, Pudjiono S, Setiadi D, Sumardi, Pujiono E. 2023. Assessing the genetic structure of teak from Southeast Sulawesi and its implication for genetic conservation and utilization in Indonesia. For Sci Technol 19 (1): 21-29. DOI: 10.1080/21580103.2023.2165281.

Oguis GK, Gilding EK, Jackson MA, Craik DJ. 2019. Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine. Front Plant Sci 10: 645. DOI: 10.3389/fpls.2019.00645.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2: 225-238. DOI: 10.1007/BF00564200.

POWO. 2025. Plant of the World Online. https://powo.science.kew.org/. Accessed 23 May 2025.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155 (2): 945-959. DOI: 10.1093/genetics/155.2.945.

Rebrean FA, Fustos A, Szabo K, Lisandru TT, Rebrean MS, Varga MI, Pamfil D. 2023. Genetic diversity and structure of *Quercus petraea* (Matt.) Liebl. populations in central and northern Romania revealed by SRAP markers. Divers 15 (10): 1093. DOI: 10.3390/d15101093.

Rini DS, Budiyanti Y, Valentine M, Permana R. 2023. ISSR and SRAP for assessing genetic variability of Indonesian local rice genotypes (*Oryza sativa* L.). Crop Breed Appl Biotechnol 23 (4): e448923411. DOI: 10.1590/1984-70332023v23n4a46.

Russo A, Mayjonade B, Frei D, Potente G, Kellenberger RT, Frachon L, Copetti D, Studer B, Frey JE, Grossniklaus U, Schlüter PM. 2022. Low-input high-molecular-weight DNA extraction for long-read sequencing from plants of diverse families. Front Plant Sci 13: 883897. DOI: 10.3389/fpls.2022.883897.

Saswita HM, Syamsuardi S, Nurainas N, Suwardi AB, Taufiq A. 2023. Phylogenetic analysis of *Baccaurea* spp. in West Sumatra using *MatK* molecular markers. Biosaintifika 15 (3): 362-369. DOI: 10.15294/biosaintifika.v15i3.46051.

Stojnić S, Avramidou EV, Fussi B, Westergren M, Orlović S, Matović B, Trudić B, Kraigher H, Aravanopoulos FA, Konnert M. 2019. Assessment of genetic diversity and population genetic structure of Norway spruce (*Picea abies* (L.) Karsten) at its southern lineage in Europe: Implications for conservation of forest genetic resources. Forests 10 (3): 258. DOI: 10.3390/f10030258.

Suarna IW, Wijaya IMS. 2021. Butterfly pea (*Clitoria ternatea* L.: Fabaceae) and its morphological variations in Bali. J Trop Biodivers Biotechnol 6 (2): jtbb63013. DOI: 10.22146/jtbb.63013.

Surya D, Rajamani K, Suresh J, Uma D. 2023. Morphological characterization and assessment of anthocyanin in three different genotypes of *Clitoria ternatea* L. Pharm Innov J 11 (7): 2388-2392.

Szabo K, Pamfil D, Bădărău AS, Hârţa M. 2021. Assessment of genetic diversity and population structure of the endangered *Astragalus exscapus* subsp. *transsilvanicus* through DNA-based molecular markers. Plants 10 (12): 2732. DOI: 10.3390/plants10122732.

Wang J, Dai W, Chen J, Ye K, Lai Q, Zhao D. 2023. Assessment of genetic diversity and genetic structure of *Saussurea medusa* (Asteraceae), a "sky island" plant in the Qinghai-Tibet Plateau, using SRAP markers. Plants 12 (13): 2463. DOI: 10.3390/plants12132463.

Wardi ES, Syukur S, Chaidir Z, Jamsari J. 2024. Genotypic identification and catechin profiling of *Uncaria gambir* in West Sumatra, Indonesia. Biodiversitas 25 (3): 1151-1158. DOI: 10.13057/biodiv/d250330.

Yan W, Li J, Zheng D, Friedman C, Wang H. 2019. Analysis of genetic population structure and diversity in *Mallotus oblongifolius* using ISSR and SRAP markers. PeerJ 7: e7173. DOI: 10.7717/peerj.7173.

Yulita KS, Atikah TD, Wardani W, Susila. 2020. Unraveling genetic variations of *Dalbergia latifolia* (Fabaceae) from Yogyakarta and Lombok Island, Indonesia. Biodiversitas 21 (2): 833-841. DOI: 10.13057/biodiv/d210251.

Yulita KS, Susila, Rachmat HH, Dwiyanti FG, Atikah TD, Subiakto A, Pratama B, Setyawati T, Wardani W, Fambayun RA, Arrofaha N, Kamal I. 2022. Population genetic of the Indonesian rosewood (*Dalbergia latifolia*) from Java and West Nusa Tenggara revealed using sequence-related amplified polymorphism. For Sci Technol 18 (4): 172-181. DOI: 10.1080/21580103.2022.2123051.

Yusuf AF, Latifah VR, Nurcahyati VI, Nurhasanah AD, Widyasari AS, Nainggolan AP, Hasibuan ARW, Hidayat MA, Karmilah, Pratiwi AD, Aurantika R, Hasanah M, Aristya GR, Arisuryanti T, Handayani NSN, Lesmana I, Daryono BS. 2025. Intra-spesific diversity of butterfly pea (*Clitoria ternatea* L.) revealed by issr with invariable its records. Biotropia 32 (1): 57-68. DOI: 10.11598/btb.2025.32.1.2362.