

GENETIC VARIABILITY IN 12 BUTTERFLY PEA (*Clitoria ternatea* L.) ACCESSIONS: A DUAL APPROACH WITH CLUSTER AND PRINCIPAL COMPONENT ANALYSIS

KERAGAMAN GENETIK 12 AKSESI TELANG (*Clitoria ternatea* L.): PENDEKATAN TERPADU DENGAN ANALISIS KLUSTER DAN KOMPONEN UTAMA

Azka Algina¹, Debby Ustari², Arif Affan Wicaksono¹, Vergel Concibido³, Agung Karuniawan^{1*}

¹ Faculty of Agriculture, Universitas Padjadjaran, Bandung 45060, Indonesia

² Faculty of Agriculture, Universitas Islam Nusantara, Bandung 40286, Indonesia

³ Sensient Colors LLC., St. Louis, MO 63106, USA

*Correspondence: agung.karuniawan@unpad.ac.id

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ABSTRACT

Understanding genetic variability is crucial for enhancing the breeding programs of butterfly pea (*Clitoria ternatea* L.), particularly in the face of the demand for improved crop varieties. This study aims to (i) evaluate the genetic variability of 12 butterfly pea accessions based on 28 agro-morphological traits and (ii) analyze the genetic relationships among these accessions. The research was conducted from December 2022 to October 2023 at the Ciparanje Experimental Field, Faculty of Agriculture, Universitas Padjadjaran. The experimental design employed a Randomized Complete Block Design (RCBD) with 12 accessions and three replications. Observations were made on 28 agro-morphological traits. Data analysis was performed using analysis of variance (ANOVA), principal component analysis (PCA), and agglomerative hierarchical clustering (AHC). ANOVA results indicated significant diversity among the 12 accessions based on 17 agro-morphological traits. PCA results showed that the first six principal components accounted for 89.1% of the total genetic variability and identified all traits as contributing factors to the genetic variability among the accessions. AHC analysis grouped the accessions into two main clusters, with Euclidean distances ranging from 1.00 to 4.00, indicating varying levels of genetic relatedness. These findings underscore the importance of genetic variability in formulating breeding strategies, particularly in the selection of parents based on targeted agro-morphological traits.

Key words: cluster analysis, Euclidean, genetic distance, high-yield, parent line.

ABSTRAK

Informasi keragaman genetik sangat penting untuk mendukung program pemuliaan tanaman telang (*Clitoria ternatea* L.), khususnya dalam menghadapi permintaan varietas unggul. Penelitian ini bertujuan untuk (i) mengevaluasi keragaman genetik dari 12 aksesi kembang telang berdasarkan 28 karakter agro-morfologi dan (ii) menganalisis hubungan genetik di

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antara aksesi-aksesi tersebut berdasarkan 28 karakter agro-morfologi. Penelitian dilaksanakan pada Desember 2022 hingga Oktober 2023 di Kebun Percobaan Ciparanje, Fakultas Pertanian, Universitas Padjadjaran. Desain eksperimen menggunakan Rancangan Acak Kelompok Lengkap (RAKL) dengan 12 aksesi dan tiga ulangan. Pengamatan dilakukan pada 28 karakter agro-morfologi. Analisis data dilakukan dengan analisis varians (ANOVA), analisis komponen utama (PCA), dan pengelompokan hierarki aglomeratif (AHC). Hasil analisis ANOVA menunjukkan bahwa 12 aksesi telang beragam secara signifikan berdasarkan 17 karakter agro-morfologi. Hasil PCA menunjukkan bahwa enam komponen utama pertama menjelaskan 89,1% dari total keragaman genetik dan mengidentifikasi 28 karakter agro-morfologi sebagai karakter yang berkontribusi terhadap keragaman genetik 12 aksesi telang. Analisis AHC mengelompokkan 12 aksesi menjadi dua kelompok utama dengan jarak Euclidean berkisar antara 1,00 hingga 4,00, mengindikasikan tingkat kekerabatan genetik yang jauh. Temuan ini menegaskan pentingnya keragaman genetik dalam merumuskan strategi pemuliaan yang efektif, terutama dalam pemilihan tetua berdasarkan karakter agro-morfologi yang ditargetkan.

Kata kunci: analisis kluster, daya hasil tinggi, Euclidean, galur tetua, jarak genetik.

INTRODUCTION

Butterfly pea (*Clitoria ternate*) is a multipurpose native plant of Indonesia. In medicine, butterfly pea flower extract functions as a diuretic, nootropic, anti-asthmatic, anti-inflammatory, analgesic, antipyretic, antidiabetic, antilipidemic, anti-rheumatic, antioxidant, and wound healing agent (Oguis et al., 2019). In cosmetics, the high anthocyanin content in butterfly pea flowers can be used as a permanent dye that does not discolor during storage and is non-toxic to skin cells (Bujak et al., 2022). Additionally, in agriculture, butterfly pea flowers serve as a natural insecticide, effective against pests like cotton and macadamia pests while being non-toxic to beneficial insects such as bees (Oguis et al., 2020). In the food industry, the leaves can be used to produce a natural green color additive, and the flowers can be used for a natural blue color additive (Ab Rashid et al., 2021). Therefore, butterfly pea is significant in various fields.

Plant breeding to develop superior butterfly pea cultivars is therefore imperative. Plant breeding aims to enhance

the genetic potential of plants for human benefit (Acquaah, 2012). Currently, butterfly pea is still regarded as a wild plant, with no reported cultivars or registered varieties in Indonesia. Research on butterfly pea (*Clitoria ternate*) in Indonesia has primarily focused on its applications in health (Rizkawati & Rizkita, 2023), beverages (Ikhwan et al., 2022), and food (Palimbong & Pariama, 2020). However, studies on the plant breeding of butterfly pea remain limited. Therefore, this study on the genetic enhancement of butterfly pea introduces a novel contribution to the body of research on this species in Indonesia.

Genetic variability serves as a fundamental pillar in plant breeding, playing an essential role in enhancing the resilience, adaptability, and productivity of crops. Amidst the challenges posed by climate change, pests, diseases, and shifting consumer preferences, the preservation and enhancement of genetic variability within plant species have become increasingly critical (Temesgen, 2021). Leveraging diverse genetic resources in Bambara groundnut is crucial for

developing high-yielding and adaptable varieties suitable for semi-arid climates (Pasipanodya et al., 2022). By exploiting a wide genetic base, plant breeders can create new varieties that not only exhibit higher yields but also withstand biotic and abiotic stresses, thereby ensuring food security and promoting sustainable agricultural practices.

The evaluation of genetic variability within and between plant populations is typically conducted using various markers. This included (i) agro-morphological, (ii) biochemical (such as allozyme), and (iii) DNA or molecular markers (Shah et al., 2023). Agro-morphological markers rely on observable traits such as flower color, seed shape, growth habits, and pigmentation (Chesnokov et al., 2020). These markers do not require advanced technology but often necessitate large land areas for field experiments. They are influenced by phenotypic plasticity, enabling the evaluation of genetic variability under varying environmental conditions and highlighting the interaction between environmental and genotypic variations (Govindaraj et al., 2015). A genetic variability evaluation using agro-morphological markers has been conducted in butterfly pea (Karuniawan et al., 2020; Suarna & Wijaya, 2021), pea (S. Singh et al., 2022), alfalfa (Bhattarai et al., 2020), and pigeon pea (Addae-Frimpomaah et al., 2021). This underscores the reliability of agro-morphological markers in evaluating genetic variability in plants.

The investigation of genetic relationships is essential for ensuring genetic variability within a population. Genetic relationships can be elucidated through a hierarchical approach, which highlights the nature of relationships

between and among sample types using standard descriptors (Osawaru et al., 2015). This method generates a dendrogram, a graphical representation that depicts the hierarchical structure of genetic interactions within clusters or groups (Gore et al., 2022). Mustapa et al. (2024) utilized a dendrogram derived from Hierarchical Clustering Analysis (HCA) based on agro-morphological traits to explain their study results. Their cluster analysis revealed that butterfly pea accessions were divided into two clusters, A and B, with a Euclidean distance of 0.27–4.65, indicating a broad range of relationships among the tested accessions. Consequently, genetic relationship analysis is valuable for elucidating genetic variability among accessions.

The significance of genetic variability studies is underscored by their implications for breeding strategies. According to Nkhoma et al. (2020), the primary objective of genetic variability studies is to identify superior parents for breeding programs. For instance, the drought-resistant soybean variety USDA-N7006, registered in the United States in 2023, originates from the hybridization of the superior USDA breeding lines TCPR01-83 and N01-11136, highlighting the role of genetic variability in breeding (Fallen et al., 2023). Therefore, a comprehensive discussion on the implications of genetic variability studies for butterfly pea breeding is crucial for the identification of superior parental lines.

This research aims to assess the genetic variability and study the genetic relationships among 12 accessions from various countries: Indonesia, Thailand, and Brazil. It is expected that this study will serve as a foundation for the butterfly pea breeding program in Indonesia.

MATERIALS AND METHODS

Genetic Materials

The research materials included 12 butterfly pea accessions originating from three countries (Table 1). These accessions have various types of flowers. Four of them are single petal, and eight of them are more than one petal (double petals).

Table 1. List of Butterfly Pea Accessions

No.	Code	Origin	Flower Type
1.	CT8.2	Thailand	Single
2.	CT8.3	Thailand	Quintuple
3.	CT12.2	Indonesia	Quandruple
4.	CT12.3	Indonesia	Quandruple
5.	CT12.3B	Indonesia	Quintuple
6.	CT12.4	Indonesia	Triple
7.	CT12.5	Indonesia	Quandruple
8.	CT12.7	Indonesia	Single
9.	CT12.7B	Indonesia	Quandruple
10.	CT24.1	Indonesia	Quintuple
11.	CT38	Brazil	Single
12.	CT51	Thailand	Single

Experimental Design

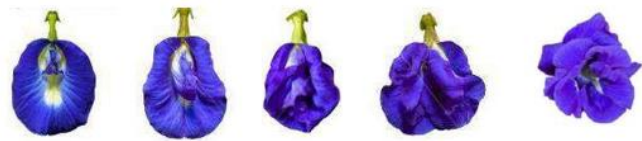
This research was designed using a Randomized Complete Block Design (RCBD) with 12 treatments (corresponding to 12 accessions) and three replications. Each treatment in each replication consisted of one hill row planted with five butterfly pea plants. The total number of plants used in each year was 165 plants. The number of samples taken was three plants from each treatment and replication.

The experiment was carried out from December 2022 to September 2023 at Ciparanje Experimental Field, Universitas Padjadjaran.

Observations

The observation variables consist of 28 agro-morphological traits, including leaf, flower, pod and seed traits. Elaboration of the traits along with their observation technique is available in Table 2.

Table 2. Observed Traits

No.	Trait	Observing Technique
1.	Leaf Length (cm)	Measured using a ruler from the top to the bottom of the leaf.
2.	Leaf Width (cm)	Measured using a ruler from the tip of the far-left leaf to the tip of the far-right leaf.
3.	Calyx Length (cm)	Measured using a ruler from the top to the bottom of the calyx.
4.	Flower Type	Observed using the scoring method shown below.
 <p>1. <i>Single petal</i> 2. <i>Double</i> 3. <i>Triple</i> 4. <i>Quandruple</i> 5. <i>Quintuple</i></p> <p>Figure 1. Scoring Method for Flower Type. Source: Shutterstock</p>		
5.	Flower Color	Observed using the Royal Horticultural Society (RHS) Color Chart.
6.	Flowering Age (Days After Planting - DAP)	Recorded when the plants produce their first flower after planting.
7.	Flower Length (cm)	Measured using a ruler from the top to the bottom of the flower.

No.	Trait	Observing Technique
8.	Flower Width (cm)	Measured using a ruler from the far-left side to the far-right side of the flower.
9.	Weight of One Fresh Flower (g)	Weighed using an analytical balance. Three flower samples were taken per plant.
10.	Fresh Flower Weight Per Plant (g)	Weighed using an analytical balance for all fresh flowers from each plant in each accession.
11.	Total Fresh Flower Weight (g)	Weighed using an analytical balance for all fresh flowers from each accession.
12.	Dried Flower Weight Per Plant (g)	Weighed using an analytical balance for all dried flowers from each plant in each accession.
13.	Total Dried Flower Weight (g)	Weighed using an analytical balance for all dried flowers from each accession.
14.	Pod Length (cm)	Measured using a ruler from the top to the bottom of the pod.
15.	Pod Width (cm)	Measured using a ruler from the far-left to the far-right end of the pod.
16.	Number of Pods Per Plant (n)	Counted the number of pods on each plant.
17.	Pod Weight Per Plant (g)	Weighed using an analytical balance for all pods from each plant in each accession.
18.	Total Number of Pods (n)	Counted the total number of pods harvested per accession.
19.	Total Pod Weight (g)	Weighed using an analytical balance for all pods from each plant in each accession.
20.	Seed shape	Observed using the scoring method illustrated below.
<p>1. <i>Kidney</i> 2. <i>Oval</i> 3. <i>Square</i> 4. <i>Elongate</i></p>		
<p>Figure 2. Scoring method for seed shape (IBPGR/ ICRISAT, 1993)</p>		
21.	Seed Color	Observed using the Royal Horticultural Society (RHS) Color Chart.
22.	Seed Length (mm)	Measured using a caliper from the top to the bottom of the seed.
23.	Seed Width (mm)	Measured using a caliper from the far-left to the far-right side of the seed.
24.	Seed Diameter (mm)	Measured using a caliper on the seed.
25.	Number of Seeds Per Pod (n)	Counted the number of seeds in each pod. Three pods were sampled for each accession.
26.	Seed Weight Per Plant (g)	Weighed using an analytical balance for the seeds produced by each plant.
27.	Total Seed Weight (g)	Weighed using an analytical balance for all the seeds produced by each accession.
28.	Weight of 100 Seeds (g)	Weighed using an analytical balance for 100 seeds produced from each accession.

Data Analysis

Analysis of variance is employed to assess the variance among accessions for the observed traits. The sources of variation in this study consist of the 12 tested butterfly pea accessions. The estimation of variation pertains to the agro-morphological traits exhibited by these accessions.

Genetic variability analysis employs multivariate techniques such as Principal Component Analysis (PCA) as proposed by (Jeffers, 1967). The analysis will be computed using the following equation:

$$PC1 = \sum_{j=1}^p a_j X_j$$

Description:

$a_j X_j$ = Linear coefficient – Eigen vectors.

The Principal Component Analysis (PCA) is employed to identify significant variables that contribute to genetic variability and to visualize their relationships with the tested accessions (Venujayakanth et al., 2017).

Cluster analysis is utilized to elucidate the genetic variability pattern among the accessions (Houmanat et al., 2021). The data is calculated using Euclidean distance and processed using the complete linkage method, and subsequently visualized in the form of a dendrogram.

Both PCA and AHC analyses were conducted using the SmartstatXL Add-in, and data interpretation was guided by explanations from the primary source website, www.smartstat.info.

RESULTS AND DISCUSSION

Analysis of Variance

Genetic variability among the 12 butterfly pea accessions based on agro-morphological traits was evaluated through

analysis of variance (ANOVA). ANOVA is a statistical technique used to compare means across multiple groups to determine if there are significant differences among them (Kim, 2017). This method is crucial for understanding variations within and between different datasets, thereby identifying the sources of variation (Acquaah, 2012). In this context, ANOVA was applied to 28 agro-morphological traits to analyze their diversity and significance among the 12 accessions.

The ANOVA results (Table 3) demonstrated the variability and significance of numerous traits among the accessions. Traits that exhibited significant differences across groups include leaf length, calyx length, flowering age, weight of a single fresh flower, fresh flower weight per plant, total fresh flower weight, dried flower weight per plant, total dried flower weight, pod length, pod width, number of pods per plant, pod weight per plant, total number of pods, total pod weight, seed weight per plant, total seed weight, and weight of 100 seeds. Conversely, traits such as leaf width, flower length, flower width, seed length, seed width, seed diameter, and number of seeds per pod did not display significant differences. Similar findings by Ulimaz et al. (2020) corroborate these results, highlighting traits like flower length, weight of a single fresh flower, total pod weight, and weight of 100 seeds as sources of variation across 38 butterfly pea accessions in different environments. The variation observed in agro-morphological traits among accessions reflects genetic differences influenced by genotype, environment, and their interaction (GE) (Acquaah, 2012; Annicchiarico, 2002). Although the current analysis does not delve into environmental and GE

interactions, agro-morphological variation is indicative of genetic variability within each accession. These findings affirm that

the 12 butterfly pea accessions exhibit genetic variability based on agro-morphological traits.

Table 3. Analysis of variance (ANOVA) on agro-morphological traits among 12 butterfly pea accessions

No.	Traits	Value		Mean	F	CV (%)
		Min	Max			
1.	Leaf Length (cm)	2.55	6.10	4.66	2.89*	13.39
2.	Leaf Width (cm)	1.95	3.75	2.96	1.75 ^{ns}	13.43
3.	Calyx length (cm)	1.00	2.6	2.21	1.83*	13.99
4.	Flowering Age (DAP)	44.00	69.67	49.01	4.70*	7.58
5.	Flower Length (cm)	2.53	4.15	3.37	0.90 ^{ns}	8.80
6.	Flower Width (cm)	2.87	5.63	4.06	1.63 ^{ns}	15.93
7.	Weight of One Fresh Flower (g)	0.32	1.25	0.65	3.71*	24.95
8.	Fresh Flower Weight Per Plant (g)	1.59	49.42	14.56	5.06*	43.64
9.	Total of Fresh Flower Weight (g)	16.51	396.28	178.28	2.57*	49.21
10.	Dried Flower Weight Per Plant (g)	0.23	4.22	1.61	5.81*	36.25
11.	Total of Dried Flower Weight (g)	2.31	82.82	29.12	5.69*	41.89
12.	Pod Length (cm)	6.00	10.97	9.52	2.28*	9.09
13.	Pod Width (mm)	0.91	1.20	1.01	1.96*	6.26
14.	Number of Pods per Plant (n)	32.5	558	211.02	2.80*	53.82
15.	Pod Weight Per Plant (g)	43.63	385.65	140.58	3.86*	47.53
16.	Total of Number Pods (n)	169	2107	739.14	15.14*	28.14
17.	Total Pod Weight (g)	95.24	1474.47	529.55	7.21*	41.26
18.	Seed Length (mm)	5.35	6.9	6.24	1.47 ^{ns}	5.34
19.	Seed Width (mm)	3.74	4.73	4.10	1.79 ^{ns}	7.01
20.	Seed Diameter (mm)	2.10	3.7	2.66	1.67 ^{ns}	10.06
21.	Number of Seeds Per Pod (n)	5.00	9.33	7.12	0.77 ^{ns}	14.19
22.	Seed Weight Per Plant (g)	6.05	148.77	48.79	6.47*	40.87
23.	Total Weight seed (g)	25.81	580.82	179.82	6.26*	39.97
24.	Weight of 100 Seeds (g)	4.16	6.46	5.30	6.42*	6.83

Note: * = significant at 0.05 probability level. ^{ns} =not significant

Genetic variability based on agro-morphological traits informs the objectives of plant breeding. Traits such as Weight of One Fresh Flower (F=3.71*) and Fresh Flower Weight per Plant (F=5.06*) demonstrate significant differences, highlighting variability in flower production and biomass (Filio et al., 2023; Ulimaz et al., 2020). Similarly, traits including Pod Length (F=2.28*), Pod Width (F=1.96*), Number of Pods per Plant (F=2.80*), and Total Pod Weight (F=7.21*) exhibit significant

differences, underscoring their importance in assessing crop productivity (Toleikiene et al., 2021). Conversely, traits like seed length and seed width do not show significant differences, but seed weight per plant (F=6.47*) and total weight of seed (F=6.26*) display significant variation, suggesting specific advantages in seed production across different groups (Akpo et al., 2020). The notable differences observed in these traits indicate distinct strengths in certain characteristics among different groups,

which can be targeted in breeding programs. For instance, Flowering Age and Pod Weight are crucial for selecting superior plants based on yield and growth rate (Killi & Beycioglu, 2022; Mallikarjuna et al., 2019). Furthermore, variations in Leaf and calyx length may indicate adaptability to varying environmental conditions (Hossain et al., 2020). The ANOVA results highlight significant variability in several pivotal traits, offering valuable insights for advancing plant breeding efforts.

Principal Component Analysis (PCA)

PCA was utilized to pinpoint the agro-morphological traits responsible for variation among the 12 butterfly pea accessions. Principal Component Analysis (PCA) is a statistical method employed to simplify high-dimensional data by transforming it into a set of uncorrelated variables termed principal components (Jolliffe & Cadima, 2016). These components capture the maximum variance in the dataset, facilitating the identification of patterns and key characteristics (Kherif & Latypova, 2019). In this context, PCA was applied to 28 agro-morphological traits (comprising four qualitative traits and 24 quantitative traits) to reduce complexity and discern the most influential variables contributing to genetic variability among the 12 butterfly pea accessions.

The principal component analysis (PCA) of agro-morphological traits for 12 butterfly pea accessions reveal significant insights. Six principal components were identified, with the first principal component (PC1) exhibiting the highest eigenvalue of 11.561, accounting for 41.3% of the total variance (Table 4). Collectively, the first six components explain 89.1% of the total

variance. In PCA, achieving a high cumulative variance (typically exceeding 80%) is advantageous as it indicates that the majority of the data's information is preserved within a few principal components (Jolliffe & Cadima, 2016; Lastovicka & Jackson, 1992; Ringnér, 2008). This facilitates data interpretation and reduces dimensionality without significant information loss. Therefore, the components PC1 through PC6 are crucial for identifying the key variables or traits.

Table 4. Eigenvalue, proportion and cumulative of each component

PC	Eigenvalue	Proportion	Cumulative
1	11.561	0.413	0.413
2	4.536	0.162	0.575
3	3.388	0.121	0.696
4	2.333	0.083	0.779
5	1.815	0.065	0.844
6	1.311	0.047	0.891

High positive loadings (≥ 0.5 or ≤ -0.5) indicate that these traits contribute significantly to the diversity among the butterfly pea accessions. PC1 to PC6 reveal that 28 agro-morphological traits significantly contribute to the observed variation (Table 5). Similarly, Gangadhara et al. (2024) performed a principal component analysis on 60 diverse Indian bean types for nine agro-morphological traits, finding that these traits accounted for 77.33% of the variation. They suggested that this genetic variability could be utilized in crossbreeding programs to develop potential segregants. Therefore, this comprehensive analysis highlights the importance of considering a wide range of agro-morphological traits, thereby enhancing the genetic improvement and utilization of butterfly pea accessions.

Table 5. Component Loadings and Communalities

Traits			PC1	PC2	PC3	PC4	PC5	PC6	Communi- nality
No.	Name	Code							
1.	Leaf Length (cm)	T1	0.436	0.322	-0.306	0.536	-0.557	0.000	0.984
2.	Leaf Width (cm)	T2	0.235	0.625	-0.340	0.297	-0.460	0.092	0.870
3.	Calyx Length (cm)	T3	-0.469	-0.006	0.697	-0.023	-0.263	0.326	0.881
4.	Flower Type	T4	0.731	0.119	0.323	-0.240	-0.228	-0.244	0.822
5.	Flower Color	T5	0.322	0.348	-0.517	-0.224	0.432	-0.300	0.819
6.	Flowering Age (DAP)	T6	-0.106	-0.846	-0.210	0.019	0.173	0.258	0.868
7.	Flower Length	T7	-0.388	-0.144	0.568	0.565	-0.063	0.206	0.860
8.	Flower Width (cm)	T8	0.596	0.164	-0.413	-0.323	0.094	0.230	0.718
9.	Weight of One Fresh Flower (g)	T9	0.911	0.078	-0.156	-0.148	-0.066	0.018	0.887
10.	Fresh Flower Weight Per Plant (g)	T10	0.927	0.190	0.142	-0.044	-0.084	-0.166	0.953
11.	Total Fresh Flower Weight (g)	T11	0.777	0.199	0.287	-0.218	-0.090	-0.007	0.781
12.	Dried Flower Weight Per Plant (g)	T12	0.911	0.248	0.111	-0.085	-0.097	-0.151	0.943
13.	Total Dried Flower Weight Per Plant (g)	T13	0.911	0.296	-0.122	0.068	-0.064	-0.058	0.946
14.	Pod Length (cm)	T14	-0.339	0.607	0.288	-0.422	-0.131	0.389	0.912
15.	Pod Width (cm)	T15	0.566	0.129	0.664	0.077	-0.263	-0.020	0.854
16.	Number of Pods Per Plant (n)	T16	-0.786	0.516	0.116	0.026	-0.057	-0.215	0.948
17.	Pod Weight Per Plant (g)	T17	-0.880	0.411	0.042	-0.034	0.183	-0.082	0.986
18.	Total Number of Pods (n)	T18	-0.869	0.355	-0.039	0.323	0.050	-0.027	0.990
19.	Total Pod Weight (g)	T19	-0.842	0.411	-0.096	0.254	0.123	-0.157	0.991
20.	Seed Shape	T20	0.588	0.091	-0.285	0.631	0.193	0.044	0.873
21.	Seed Color	T21	-0.653	0.122	0.371	-0.484	0.013	-0.334	0.925
22.	Seed Length (mm)	T22	0.253	0.597	0.357	0.019	0.441	0.329	0.851
23.	Seed Width (mm)	T23	0.600	0.535	-0.144	-0.045	0.124	0.297	0.773
24.	Seed Diameter (mm)	T24	0.312	0.377	0.354	0.529	0.499	-0.227	0.945
25.	Number of Seeds Per Pod (n)	T25	-0.470	0.198	-0.627	-0.142	-0.058	0.409	0.844
26.	Seed Weight Per Plant (g)	T26	-0.509	0.700	-0.158	-0.229	-0.168	-0.034	0.856
27.	Total Seed Weight (g)	T27	-0.716	0.577	-0.181	-0.001	-0.060	-0.040	0.884
28.	Weight of 100 Seeds (g)	T28	0.640	0.353	0.372	-0.014	0.487	0.264	0.979
Expl. Variance			11.561	4.536	3.388	2.333	1.815	1.311	24.944
% Variance			0.413	0.162	0.121	0.083	0.065	0.047	
% Cum. Variance			0.413	0.575	0.696	0.779	0.844	0.891	

PCA result identified traits contributing to variation that were not detected by ANOVA. In Table 3, seven traits were not significant, including Leaf Width, Flower Length, Flower Width, Seed Length, Seed Width, Seed Diameter, and Number of Seeds Per Pod. However, in the PCA analysis, these seven traits showed high positive loadings (≥ 0.5 or ≤ -0.5), indicating that they contribute to the variation among the 12 butterfly pea accessions. A similar finding was reported by Ustari et al. (2023), where two traits in purple-fleshed sweet potato—storage root length and diameter, and number of roots per plant—were not significant in ANOVA but had high positive loadings in PCA. Samyuktha et al. (2017)

also found that Plant Width in chickpea was not significant in ANOVA, but had a positive loading (0.712) in PCA. These findings suggest that PCA can capture traits that are not detected by ANOVA.

Agglomerative Hierarchical Clustering (AHC)

Agglomerative Hierarchical Clustering (AHC) was utilized to analyze agromorphological traits, facilitating the grouping of accessions based on their similarities and differences in these traits. This method provides insights into the genetic relationships and diversity among the accessions (Houmanat et al., 2021; Murtagh & Contreras, 2012). The resulting

dendrogram visually represents these relationships, helping to identify distinct clusters of accessions. Harouna et al. (2020) employed AHC to detect genetic distances among unexplored wild *Vigna* legumes based on agro-morphological traits. Similarly, the genetic relationships of *Phaseolus vulgaris* have been elucidated using AHC (Kouam et al., 2023). This analysis has also been used to study genetic relationships in common bean (Ambachew et al., 2023), Ethiopian chickpea (Admas et al., 2021), winged bean (Singh et al., 2019) and butterfly pea (Aziza et al., 2021; Karuniawan et al., 2017; Ulimaz et al., 2020). These studies indicate that AHC is a widely used method for elucidating genetic relationships in legumes.

The dendrogram derived from the AHC analysis illustrates the clustering patterns of the 12 butterfly pea accessions based on their agro-morphological traits. It reveals the formation of distinct clusters, highlighting the genetic variability among the accessions (Figure 4). Accessions that are closely linked in the dendrogram exhibit high similarity in their agro-morphological traits, indicating closer genetic relationships. The AHC analysis results in the grouping of the 12 butterfly pea accessions into two primary clusters with subclusters, as shown in Figure 4. Cluster I comprise four accessions: CT51, CT38, CT8.2, and CT12.7. Cluster II includes the remaining eight accessions: CT12.3B, CT8.3, CT12.2, CT12.4, CT12.7B, CT12.3, and

CT12.5. Additionally, Cluster II is further divided into two subclusters: Subcluster IIA and Subcluster IIB. Accessions grouped within the same cluster exhibit high genetic similarity. Thus, the resulting dendrogram effectively delineates the genetic relationships among the 12 butterfly pea accessions based on their agro-morphological traits.

The genetic relationships among the 12 butterfly pea accessions are relatively distant. These relationships can be quantified using Euclidean distance, which measures the 'straight line' distance between two points in n-dimensional space. For these accessions, Euclidean distances range from 1.00 to 4.00, with a distance coefficient greater than 1 indicating a distant genetic relationship (Zhang et al., 2017). Aziza et al. (2021) found that the cluster analysis of 12 butterfly pea accessions showed distant relationships, with Euclidean distances ranging from 3.01 to 6.83 based on flower characteristics. Similarly, Havananda & Luengwilai (2019) reported that the dendrogram of 46 butterfly pea accessions exhibited 80% dissimilarity based on antioxidant and phytochemical activities in the flowers. Ulimaz et al. (2020) observed Euclidean distances ranging from 0.01 to 3.99 in 38 butterfly pea accessions based on agro-morphological traits. These findings underscore the significance of Euclidean distance as a crucial parameter for inferring genetic relationships among accessions.

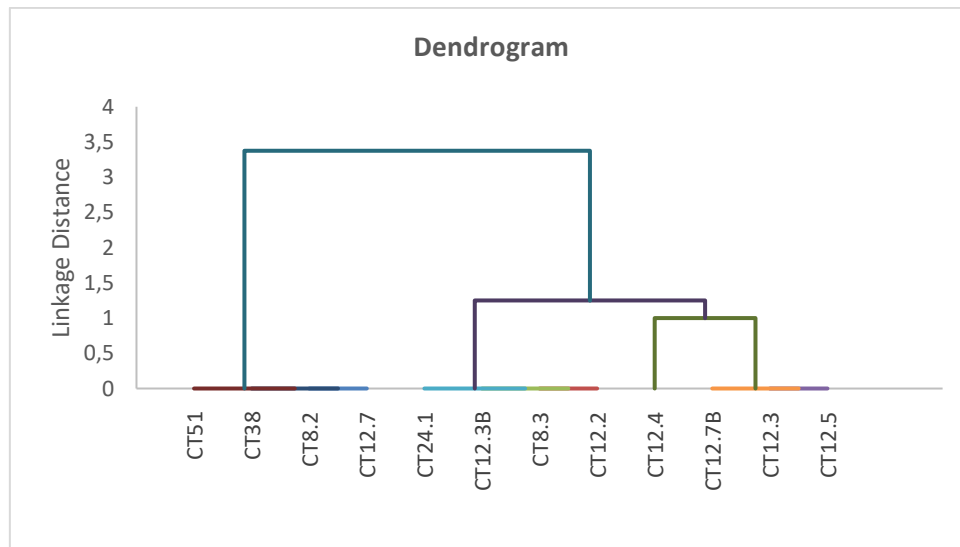


Figure 3. Dendrogram comprising 12 accessions was constructed based on 28 agro-morphological traits using Agglomerative Hierarchical Clustering (AHC)

Understanding the clustering patterns enables breeders to select parent accessions from different clusters to maximize genetic variability in the progeny. Crossing parents with distant genetic relationships has the potential to produce superior offspring. For example, in 2021, the Arkansas Agricultural Experiment Station developed the soybean variety R14-1422, which exhibits high yield potential and moderate resistance to southern root-knot nematode (Ravelombola et al., 2023). This variety resulted from crossing two genetically distant parents: R06-4433, a cultivar released by Arkansas, and the Missouri cultivar S05-11482. Thus, understanding the genetic relationships among accessions has significant implications for breeding strategies aimed at producing superior hybrids.

CONCLUSION

1. The 12 butterfly pea accessions showed significant genetic variability based on 17 agro-morphological traits according to the ANOVA results.

However, the PCA results indicated that the 12 butterfly pea accessions were significantly diverse based on 28 agro-morphological traits.

2. The PCA results complement the ANOVA results by identifying traits that were not detected by ANOVA. The genetic relationships among accessions also exhibit relatively distant with Euclidean distances ranging from 1.00 to 4.00.
3. This information is highly valuable for selecting parental candidates for artificial hybridization with specific traits.

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