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Genetic diversity and population structure of Indonesia pea (*Pisum sativum L.*) landraces based on morphological markers

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Abstract. To support pea breeding programs in Indonesia, where domestic production fails to meet demand, this study aimed to characterize the genetic diversity of local landraces. We assessed 82 pea accessions from across Indonesia using 24 qualitative morphological traits. The Shannon diversity index ranged from 0.07 to 1.45, indicating considerable variation. Principal component analysis (PCA) revealed that the first seven components (eigenvalue >1) accounted for 80.45% of the total variation. The scatter plot of PC1 and PC2 clearly separated accessions with white flowers from those with purple flowers. Cluster analysis using a Neighbor-Joining tree identified three major clusters. Bayesian population structure analysis revealed five distinct subpopulations (K=5) and identified nine admixed accessions. Analysis of Molecular Variance (AMOVA) indicated that most genetic variation (85%) resided within subpopulations rather than among them (15%). Crucially, both clustering and population structure analyses showed no clear separation based on geographical origin, suggesting significant historical seed exchange. This study provides a foundational understanding of Indonesia's pea germplasm and identifies specific, diverse accessions that can be directly utilized in breeding programs to develop high-yielding varieties adapted to abiotic and biotic stresses. **Keywords:** Pisum sativum, genetic diversity, landrace, morphological marker, population structure, Indonesia

1. Introduction

Pea (*Pisum sativum* L.) is a self-pollinating diploid plant (2n=14) belongs to family Fabaceae. Pea is one of the six major pulse crops in the world along with dry beans, chickpea, pigeon pea, lentils, and cowpea (Ray et al., 2023). Pea are categorized based on their intended purpose, either as field peas or green peas. Field pea is cultivated primarily for the purpose of harvesting dried seeds for consumption or as fodder, while green pea is cultivated for its immature seeds or tender pods, which are used as vegetables (Zhang et al., 2016). As a cool-season crop, pea are commonly cultivated in temperate or subtropical regions and are poorly suited to tropical lowlands due to susceptibility to heat and drought stress (Zanetta et al., 2024). However, they can be grown successfully in highland areas with cooler temperatures (13-18°C) (Zaki et al., 2017).

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In Indonesia, a tropical country, pea cultivation is centered in highlands such as Ngadisari-Probolinggo Regency (2000 m.asl), Berastagi-Karo Regency (1330 m.asl), Bandungan-Semarang Regency (1100 m.asl), Cilawu-Garut Regency (890 m.asl), Parakan-Temanggung Regency (820 m.asl), and Junrejo-Batu City (620 m.asl) (Waluyo et al., 2019). Despite local production, Indonesia remains a significant importer of peas, with 1,087 tonnes of dry peas and 1,508 tonnes of green peas imported in 2022, compared to minimal exports (FAOSTAT, 2022). This highlights a critical need to enhance domestic pea production.

Indonesia possesses diverse pea landraces distributed across Java and Sumatra (Waluyo et al., 2019). Landraces (known as traditional varieties/farmer varieties/local varieties) are dynamic populations that genetically heterogeneous resulting from evolutionary prosess through natural and human selection that locally adapted to specific agro-climate conditions associated with traditional cultivation systems (Nadeem et al., 2021; Caramante et al., 2024). Landraces are dynamic, genetically heterogeneous populations shaped by natural and human selection, offering valuable germplasm for breeding programs aimed at improving yield, quality, and stress tolerance (Sultana et al., 2010; Marone et al., 2021; Zanetta et al., 2023; Bagheri et al., 2023; Lazaridi et al., 2024). To reduce import dependency and expand cultivation into Indonesia's vast tropical lowlands, leveraging the genetic potential of these locally adapted landraces is crucial. They may harbor unique traits for stress tolerance.

Evaluating genetic diversity and population structure is a critical first step for efficient germplasm utilization in breeding (Mbasani-Mansi et al., 2019). Morphological markers, particularly qualitative traits, are a cost-effective and straightforward initial screening method because they are easy to score and less influenced by the environment than quantitative traits (Jansky et al., 2015; Yeshitila et al., 2024) Therefore, this study employed qualitative morphological markers to provide a foundational assessment. This study was designed to evaluate the genetic diversity and population structure of 82 Indonesian pea landraces. The generated information is vital for optimizing germplasm management and identifying promising parents for future breeding programs aimed at developing climate-resilient pea varieties for Indonesia.

2. Method

2.1 Plant Material

The study used 82 pea accessions, comprising 80 landraces from the collection of the Faculty of Agriculture, Brawijaya University, and two commercial varieties (Taichung and Berastagi) as checks. The landraces were collected from seven regencies/cities in Indonesia (Probolinggo, Batu, Boyolali, Semarang, Temanggung, Garut, and Karo) (Table 1).

Table 1. List of pea (Pisum sativum L.) accessions and origin

No	Accession	Origin	No	Accession	Origin
1	PSAT57001	Boyolali	41	PSAT56006	Temanggung
2	PSAT57003	Boyolali	42	PSAT56008	Temanggung
3	PSAT57004	Boyolali	43	PSAT56010	Temanggung
4	PSAT57005	Boyolali	44	PSAT56011	Temanggung
5	PSAT57006	Boyolali	45	PSAT56012	Temanggung
6	PSAT57007	Boyolali	46	PSAT56013	Temanggung
7	PSAT65001	Batu	47	PSAT56014	Temanggung
8	PSAT65002	Batu	48	PSAT56015	Temanggung
9	PSAT65003	Batu	49	PSAT56016	Temanggung
10	PSAT65004	Batu	50	PSAT56017	Temanggung
11	PSAT22001	Karo	51	PSAT56019	Temanggung
12	PSAT22003	Karo	52	PSAT56020	Temanggung
13	PSAT22006	Karo	53	PSAT56021	Temanggung
14	PSAT67002	Probolinggo	54	PSAT56022	Temanggung
15	PSAT67003	Probolinggo	55	PSAT56023	Temanggung
16	PSAT67006	Probolinggo	56	PSAT56024	Temanggung
17	PSAT50003	Semarang	57	PSAT56026	Temanggung
18	PSAT50005	Semarang	58	PSAT56027	Temanggung
19	PSAT50006	Semarang	59	PSAT56029	Temanggung
20	PSAT50007	Semarang	60	PSAT56031	Temanggung
21	PSAT50008	Semarang	61	PSAT56032	Temanggung
22	PSAT50009	Semarang	62	PSAT56033	Temanggung
23	PSAT50010	Semarang	63	PSAT56034	Temanggung
24	PSAT50011	Semarang	64	PSAT56035	Temanggung
25	PSAT50013	Semarang	65	PSAT44003	Garut
26	PSAT50014	Semarang	66	PSAT44004	Garut
27	PSAT50016	Semarang	67	PSAT44008	Garut
28	PSAT50017	Semarang	68	PSAT44011	Garut
29	PSAT50018	Semarang	69	PSAT44013	Garut
30	PSAT50019	Semarang	70	PSAT44014	Garut
31	PSAT50022	Semarang	71	PSAT44015	Garut
32	PSAT50023	Semarang	72	PSAT44016	Garut
33	PSAT50024	Semarang	73	PSAT44018	Garut
34	PSAT50025	Semarang	74	PSAT44023	Garut
35	PSAT50026	Semarang	75	PSAT44026	Garut
36	PSAT50027	Semarang	76	PSAT44028	Garut
37	PSAT56001	Temanggung	77	PSAT44030	Garut
38	PSAT56002	Temanggung	78	PSAT44031	Garut
39	PSAT56003	Temanggung	79	PSAT44032	Garut
40	PSAT56004	Temanggung	80	PSAT44033	Garut

2.2 Experimental Design

The experiment was conducted from 2023-2024 at an experimental field in Malang Regency (7°56'31.2"S 112°35'18.4"E). An Augmented Block Design was used with four blocks. Each block contained 22 plots (20 landraces and the two commercial checks). Each accession was planted in a single-row plot of 1 m length, 15 cm between plants within rows and 1 m between rows.

2.3 Morphological Evaluation and Data Collection

Observations were carried out at specific growth stages (e.g., flowering for flower traits, maturity for pod and seed traits) by two independent researchers to minimize subjectivity. Twenty-four qualitative morphological traits were evaluated according to the UPOV (2009) descriptor. Each

trait was scored using specific codes (Table 2), which were subsequently converted into binary data (1 = presence, 0 = absence) for analysis.

Table 2. List of qualitative morphological traits along with their traits characteristic and code

Traits name	Traits characteristics and code
Antocyanin coloration of plant	1: Absent, 9: Present
Anthocyanin coloration of axil	1: Absent, 2: Single ring, 3: Double ring
Color of foliage	1: Yellow green, 2: Green, 3: Blue green
Leaflets	1: Absent, 9: Present
Stem fascination	1: Absent, 9: Present
Position of broadest part of leaflet	1: At middle or slightly towards base, 2: Moderately towards base, 3: Strongly towards base
Flecking of stipules	1: Absent, 9: Present
Density of flecking of stipules	1: Very sparse, 3: Sparse, 5: Medium, 7: Dense, 9: Very dense
Color of wing	1: White with pink blush, 2: Pink, 3: Reddish purple
Color of standard	1: White, 2: Whitish cream, 3: Cream
Shape of apex of upper sepal	1: Acuminate, 2: Acute, 3: Rounded
Shape of base of standard	1: Strongly raised, 3: Moderately raised, 5: Level, 7: Moderately arched, 9: Strongly arched
Parchment of pod	1: Absent or partial, 9: Entire
Thicktened wall of pod	1: Absent, 9: Present
Shape of distal part of pod	1: Pointed, 2: Blunt
Curvature on pod	1: Absent or very weak, 3: Weak, 5: Medium, 7: Strong, 9: Very strong
Color of pod	1: Yellow, 2: Green, 3: Blue green, 4: Purple
Shape of seed	1: Ellipsoid, 2: Cylindrical, 3: Rhomboid, 4: Irrengular
Wrinkling of cotyledon	1: Absent, 9: Present
Color of cotyledon	1: Green, 2: Yellow, 3: Orange
Violet or pink spots on testa	1: Absent, 2: Fair, 3: Intense
Marbling of testa	1: Absent, 9: Present
Hilum color	1: Same color as testa, 2: Darker than testa
Color of testa	1: Reddish brown, 2: Brown, 3: Brownish green

2.4 Data analysis

The morphological diversity of the accessions was initially quantified by calculating the Shannon diversity index (H′) for each trait using the formula $H' = -\sum_{i=1}^{R} P_i ln(P_i)$, where Pi is the proportion of observations in the ith category (Nolan and Callan, 2006). A subsequent Principal Component Analysis (PCA) was performed using XLSTAT, based on a Pearson correlation matrix, to reduce data dimensionality. Components with an eigenvalue ≥1.0 were retained, and traits were considered significant contributors if their loadings were ≥|0.60| (Peres-Neto et al., 2003). For assessing genetic relationships, genetic distance was estimated using Jaccard's coefficient, followed by the construction of a Neighbor-Joining (NJ) dendrogram with MEGA11 software (Tamura et al., 2021). The underlying population structure was investigated via Bayesian clustering in STRUCTURE v2.3.4 (Pritchard et al., 2000), employing an admixture model with K (number of populations) ranging from 1 to 15, a burn-in period of 10,000, and 10,000 MCMC repetitions. The optimal K was determined using the ΔK method (Evanno et al., 2005) via the online web-based STRUCTURE HARVESTER program (Earl and VonHoldt, 2012), and individuals were assigned to a subpopulation if their membership coefficient was ≥0.8, otherwise they were considered admixed (Singh et al., 2016). Finally, Analysis of Molecular Variance (AMOVA) was conducted using binary matrices of morphological data in GenAlEx 6.5 (Peakall and Smouse,

2012) to partition the genetic variance among and within the subpopulations identified by the STRUCTURE analysis.

3. RESULTS

3.1 Morphological Characterization

Among the 24 qualitative traits evaluated, four were monomorphic (leaflets, stem fascination, flecking of stipules, and pod color), while the remaining 20 were polymorphic. Anthocyanin coloration was present in 90.2% of accessions, predominantly from Boyolali, Semarang, Batu, Garut, and Temanggung. Accessions from Probolinggo, Karo, and the two commercial checks lacked anthocyanin. Most accessions had acuminate sepal apices (81.7%) and a level standard base (85.4%). All pods were green, with 57.3% being blunt-shaped. Most accessions (82.9%) had edible pods (absent parchment), and almost all (96.3%) had wrinkled cotyledons. Cotyledon color was primarily yellow (79.3%), and testa color varied, with brown (37.8%) and brownish green (32.9%) being most common. Figure 1 shows the variation in the color characteristics of pea flowers.



Figure 1. Flower color variation of Indonesia pea landraces

3.2 Shannon Diversity Index

The Shannon diversity index for the 20 polymorphic traits ranged from 0.07 (color of foliage, thickened wall of pod, seed shape) to 1.45 (density of flecking of stipules), with a mean of 0.49 (Table 3). Traits related to seed (color of testa: 1.28) and pod (curvature on pod: 0.82) showed high diversity.

Table 3. Shanon diversity index of 20 morphological traits

Traits	Diversity Index
Anthocyanin coloration of plant	0.32
Anthocyanin coloration of axil	0.32
Color of foliage	0.07
Position of broadest part of leaflet	0.42
Density of flecking of stipules	1.45
Color of wing	0.47
Color of standard	0.32
Shape of apex of upper sepal	0.48
Shape of base of standard	0.48

Parchment of pod	0.46
Thicktened wall of pod	0.07
Shape of distal part of pod	0.68
Curvature on pod	0.82
Shape of seed	0.07
Wrinkling of cotyledon	0.16
Color of cotyledon	0.56
Violet or pink spots on testa	0.66
Marbling of testa	0.19
Color of hilum	0.60
Color of testa	1.28

3.3 Principal component analysis (PCA)

The first seven PCs had eigenvalues >1 and collectively explained 80.45% of the total variation. PC1 (32.42%) was strongly positively associated with anthocyanin-related traits (plant, axil, wing) and seed traits (violet/pink spots on testa, testa color) and negatively associated with the position of the broadest part of the leaflet and standard color (Table 4). This indicates that flower and seed pigmentation are major sources of variation. PC2 (14.45%) was influenced by pod wall thickness, seed shape, and foliage color. The scatter plot of PC1 and PC2 (Figure 2) showed a clear separation between white-flowered accessions (Karo, Probolinggo, checks) and purple-flowered accessions (other regions), highlighting the discriminatory power of these traits.

Table 4. Principal component analysis of 20 morphological traits

Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Antocyanin coloration of plant	**0.94**	0.24	0.13	0.05	0.03	0.04	0.07
Antocyanin coloration of axil	**0.94**	0.24	0.13	0.05	0.03	0.04	0.07
Color of foliage	-0.59	**0.75**	0.11	0.11	0.15	-0.09	0.12
Position of broadest part of leaflet	**-0.73**	-0.31	0.16	0.19	0.08	-0.13	0.13
Density of flecking of stipules	-0.31	-0.20	**0.68**	-0.06	0.10	0.00	-0.19
Shape of apex of upper sepal	-0.11	0.39	0.23	-0.29	-0.26	0.01	-0.41
Color of standard	**-0.94**	-0.24	-0.13	-0.05	-0.03	-0.04	-0.07
Shape of base of standard	-0.32	-0.17	-0.30	-0.38	0.09	0.38	0.45
Color of wing	**0.93**	0.24	0.13	0.06	0.05	0.02	0.07
Curvature on pod	-0.27	0.55	-0.14	0.26	-0.28	0.29	-0.09
Shape of distal part of pod	0.25	0.00	-0.50	0.42	0.36	-0.11	-0.04
Thicktened wall of pod	-0.59	**0.75**	0.11	0.11	0.15	-0.09	0.12
Parchment of pod	0.20	0.07	-0.48	-0.28	**0.64**	-0.24	-0.08
Shape of seed	-0.59	**0.75**	0.11	0.11	0.15	-0.09	0.12
Color of cotyledon	0.24	-0.41	0.34	**0.63**	0.03	-0.12	0.18
Wrinkling of cotyledon	-0.09	-0.05	-0.07	0.35	0.37	0.35	**-0.63**
Violet or pink spots on testa	**0.63**	0.13	0.38	0.04	0.15	-0.24	0.09
Marbling of testa	0.18	0.08	0.25	0.05	0.30	**0.71**	0.19
Color of testa	**0.69**	0.33	-0.12	-0.41	-0.09	-0.04	-0.14
Color of hilum	0.25	0.17	-0.55	0.45	-0.47	0.02	0.03
Eigenvalue	6.48	2.89	1.87	1.53	1.26	1.04	1.00
Variance (%)	32.42	14.45	9.37	7.68	6.30	5.21	5.02

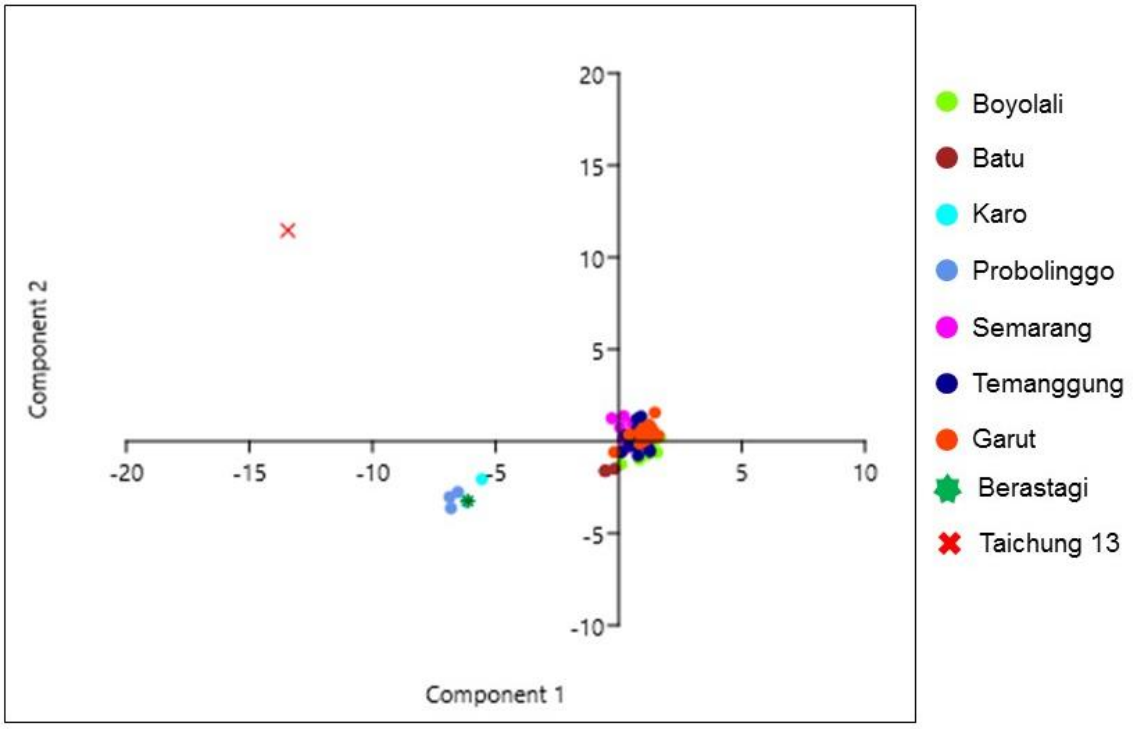


Figure 2. Scatter plot in PCA showing distribution of 82 pea accessions based on PC1 and PC2

3.4 Genetic Distance and Cluster Analysis

The genetic distance based on Jaccard's coefficient ranged from 0.00 (between 15 identical pairs) to 0.83. The NJ dendrogram grouped the 82 accessions into three major clusters (Figure 3). Cluster I consisted of white-flowered accessions from Karo, Probolinggo, and the two commercial checks. Clusters II and III comprised purple-flowered accessions from Boyolali, Batu, Semarang, Temanggung, and Garut. Cluster III was subdivided into four subclusters (IIIA, IIIB, IIIC, IIID). Notably, geographical origin was not a reliable predictor of genetic grouping, as accessions from the same region (e.g., Temanggung) were distributed across different clusters.

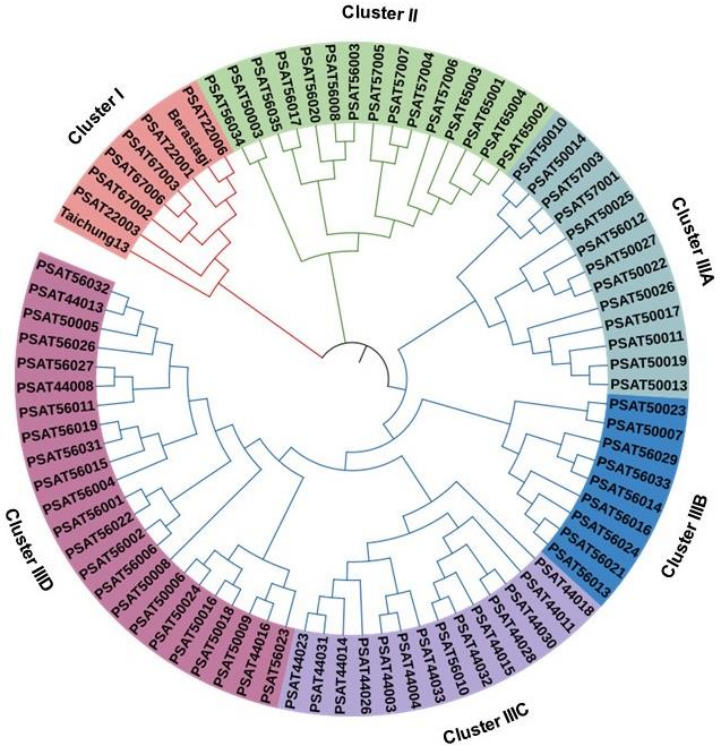


Figure 3. Neighbor joining tree dendogram of 82 pea accessions based on the genetic distance of morphological traits

3.5 Population structure and AMOVA

The Evanno's method indicated the optimal number of subpopulations (K) was five (Figure 4). The population structure analysis (Figure 5) revealed that 89% of accessions were pure (membership ≥0.8), while nine accessions (11%) were admixed. The grouping pattern was largely consistent with the NJ tree. Subpopulation 1 corresponded to Cluster II, Subpopulation 2 to parts of Cluster III, Subpopulation 3 to Cluster IIIC (dominated by Garut accessions with pod parchment), Subpopulation 4 to Cluster I, and Subpopulation 5 to the remaining accessions in Cluster IIID. AMOVA confirmed that the majority of genetic variation (85%) existed within subpopulations, while only 15% was found among subpopulations (PhiPT = 0.147, $p < 0.001$) (Table 5).

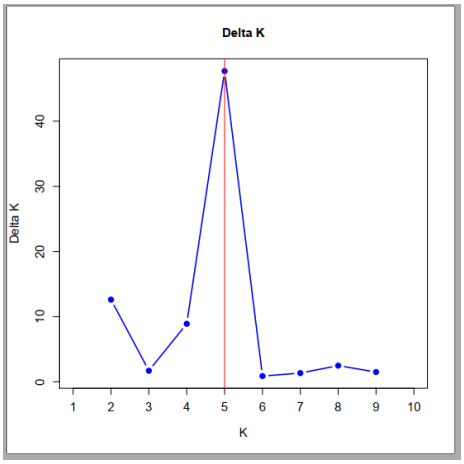


Figure 4. Estimated number of subppulation (K), peak at K = 5

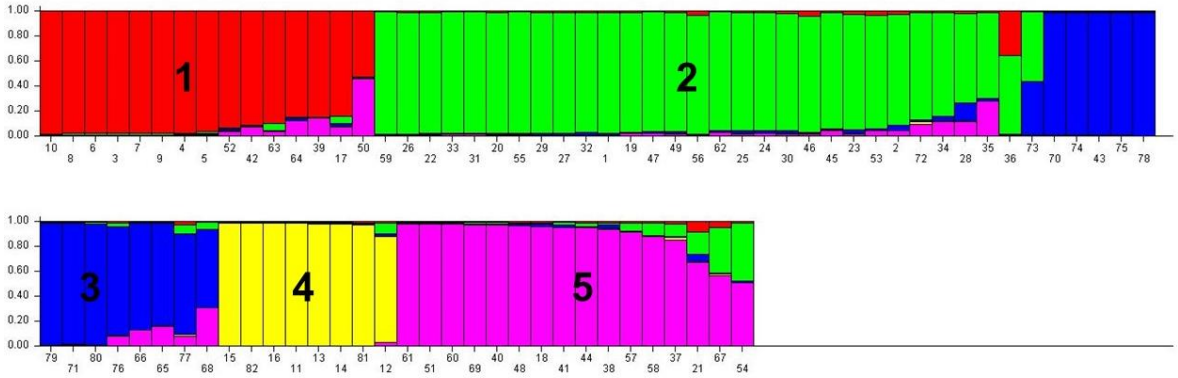


Figure 5. Population structure of 82 pea accessions based on 24 morphological markers.

Table 5. Analysis of molecular variance of 82 pea accessions based on 24 morphological markers

Source	df	SS	MS	Est. Var.	%
Among Subpopulation	4	73.525	18.381	0.859	15%
Within Subpopulation	77	384.390	4.992	4.992	85%
Total	81	457.915		5.851	100%

4. Discussion

Our comprehensive assessment reveals substantial morphological diversity within Indonesian pea landraces, organized into distinct genetic groups without a strong geographical pattern. The high genetic variation observed within subpopulations is particularly advantageous for breeding, as it suggests that valuable alleles can be found within local populations.

The high Shannon index for traits like stipule flecking and testa color aligns with findings in other pea diversity studies (Sharma et al., 2022). The PCA clearly identified flower color and anthocyanin pigmentation as the primary factors differentiating accessions, consistent with reports that these traits are highly heritable and less influenced by the environment (Hanci and Cebeci, 2019; Mohamed et al., 2019). This makes them reliable markers for preliminary classification and selection.

The lack of correlation between genetic clustering and geographical origin has been observed in other legume studies (Lombardi et al., 2014; Liu et al., 2020; Özkan et al., 2022). This can be attributed to long-standing seed exchange among farmers across different regions, leading to a sharing of genetic backgrounds and creating admixed genotypes (Jain et al., 2014; Rana et al., 2017; Joshi et al., 2023). This finding implies that future germplasm collection missions should focus on phenotypic diversity within a location rather than solely targeting distant geographical areas.

The AMOVA result, showing high within-population variation (85%), is consistent with studies on other legumes like garden pea (Sharma et al., 2022), cowpea (Zafeiriou et al., 2023), and faba bean (Avramidou et al., 2023). This pattern is typical for self-pollinating species where local populations can maintain high heterogeneity, especially in landraces (Mohamed et al., 2019). For plant breeders, this means that intense selection within a promising landrace can yield significant genetic gain.

The practical implications of this study are twofold. First, the identification of distinct subpopulations, such as the Garut group (Subcluster IIIC) with unique pod parchment, provides direct targets for breeders seeking specific traits. Second, the high within-population diversity indicates that individual accessions from any location contain a rich genetic reservoir. This enables effective selection within local populations for complex traits like yield stability and abiotic stress tolerance. Future work should integrate molecular markers with these morphological data to pinpoint specific genes underlying the observed variation, particularly for traits like anthocyanin production and drought tolerance, accelerating the development of superior pea varieties for Indonesian agriculture.

5. Conclusion

This study demonstrates that Indonesian pea landraces possess significant genetic diversity, structured into five subpopulations with a high degree of admixture and no strong geographical patterning. The variation is predominantly found within subpopulations. Key morphological traits, especially related to flower color and seed characteristics, are effective for differentiating accessions. These findings provide a solid foundation for the conservation and utilization of Indonesian pea germplasm. The identified subpopulations and diverse accessions represent valuable genetic resources for breeding programs aimed at developing improved pea varieties with enhanced yield and adaptability to tropical environments.

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