

Genetic diversity of local cowpea (*Vigna unguiculata*) germplasms of East Nusa Tenggara Province, Indonesia revealed by seed characteristics

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Manuscript received: 26 November 2024. Revision accepted: 12 March 2025.

Abstract. Mau YS, Oematan SS, Arsa IGBA, Ndiwa ASS, Bere AC. 2025. Genetic diversity of local cowpea (*Vigna unguiculata*) germplasms of East Nusa Tenggara Province, Indonesia revealed by seed characteristics. Biodiversitas 26: 1279-1288. Cowpea (*Vigna unguiculata*) is one of the predominant pulse crops in the tropical and sub-tropical regions, including Indonesia. Cowpea is commonly cultivated in a mixed cropping system with maize by dryland farmers in West Timor, East Nusa Tenggara Province, Indonesia. Still, information about its genetic diversity level is unavailable. This study aimed to characterize the cowpea seeds from West Timor and to determine the genetic diversity based on seed characteristics. A total of 126 seed accessions were collected from farmers in the district of production centers in five districts in West Timor. The collected seeds were observed for qualitative and quantitative characteristics in the laboratory. The data were subjected to cluster analysis to determine the genetic diversity level and principal component analysis to determine the characters mostly responsible for diversity. The results showed a high genetic diversity among the accessions. Cluster analysis results revealed that the collected accessions were grouped into three clusters at a truncation point of 8.0, corresponding with seed characteristics. Four main principal components (PC) explained most (81.70%) of the observed variability in the data set. Both seed morphological and quantitative characters contributed almost equally to the diversity of the accessions.

Keywords: Cowpea, East Nusa Tenggara Province, genetic diversity, seed characterization, *Vigna unguiculata*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is widely grown in tropical and sub-tropical regions for its high adaptability to various climatic conditions and cropping systems. Cowpea enhances soil nitrogen content through its symbiosis with soil microorganisms (Setyowati and Minantyorini 2016; Trustinah et al. 2017). The cowpea is rich in carbohydrates, protein, fat (Gerrano et al. 2019; FAOSTAT 2021; Abebe and Alemayehu 2022), vitamins, minerals (Enyiukwu et al. 2018; Mekonnen et al. 2022), and polyphenols with high antioxidant properties (Lim et al. 2021; Damián-Medina et al. 2022).

Cowpea is cultivated by farmers in Indonesia as a plant-based protein source due to its high protein content, rendering it an alternative raw material to produce processed food called *tempe* (Rahmawati et al. 2021). In West Timor, East Nusa Tenggara (ENT) Province, cowpea is cultivated in a mixed cropping system with other crops. This cropping system includes up to three crops, such as maize, cowpea, and pumpkin called *salome* or *t-sen tabua* (Dimu-Heo et al. 2022). This system is similar to that in Mexico called *milpa* (Palacios-Rojas 2020; Novotny et al. 2021), and *three sisters* mostly adopted in Central and South American countries (Mt. Pleasant 2016; Ngapo et al. 2021).

Mixed cropping is prone to high competition among mixed crops for growth resources. Thus, the yield of each crop component is lower than its monoculture system. The Indonesian cowpea harvest area in 2019 was 702.163 ha with a total production of 826.351 tons and productivity of

1.17 t ha⁻¹ (Ministry of Agriculture 2019). There is no formal record of total cowpea production in ENT, but its productivity in the *salome* system is 0.623 t ha⁻¹ (Nendissa et al. 2023). In the *salome* system utilizing local crop species, the competition for light is relatively small compared to that in the root zone. This is because the morphology of the three components is arranged in the upper, middle, and lower strata, where maize occupies the upper strata due to its higher habitus, cowpea occupies the middle strata with its stems to wrap around the maize stalks, and pumpkin occupies the lower strata as the crop creeps on the soil surface (Dimu-Heo et al. 2022). In practice, however, the use of improved maize varieties affects the composition of the above strata. The currently released superior maize varieties tend to have a shorter habitus than the local ones.

Several superior cowpea varieties in Indonesia include KT-1, KT-7, KT-9, Albina IPB, Arghavan IPB, UNO IPB, and Tampi IPB (Wahyudi et al. 2022). However, these varieties are still unavailable at the farmer's level in West Timor. The cowpea varieties used in the *salome* system in West Timor are still the local ones, with different local names, that are able to creep to the tip of the corn stalk or even beyond causing a high competition for sunlight in the upper strata. Thus, it is vital to make a balanced arrangement among strata to reduce competition. This can be done by planting maize and cowpea varieties with different habitus so that they can be included in the *salome* system with a low level of upper strata competition. The availability of cowpeas varieties with varying habitus allows their use in various planting systems.

ENT is rich in local cowpea germplasm but data on the number of local genotypes and genetic diversity is unavailable. Local germplasm of ENT was studied by Hosang et al. (2015) and Setyowati and Minantyorini (2016), but the scope was limited in the study area and collection size. Information on the cowpea germplasm genetic diversity is valuable to designing a more efficient growth resource utilization for *salome* system and as parental sources for the development of superior cowpea varieties. This study aimed to determine the genetic diversity of cowpea germplasm of ENT Province, Indonesia based on seed characteristics and to determine the factors mostly responsible for the diversity.

MATERIALS AND METHODS

Research site

The study was conducted in two stages: (i) involving a survey method in the farmer's field and (ii) seed morphological and quantitative character observation in the laboratory. The survey was conducted in five districts in West Timor, East Nusa Tenggara Province, Indonesia, i.e., Kupang, Timor Tengah Selatan (TTS), Timor Tengah Utara (TTU), Belu, and Malaka Districts. The laboratory observation was carried out in the Seed Technology Laboratory, Faculty of Agriculture, Universitas Nusa Cendana, Kupang, Indonesia. The survey and seed collection were carried out during the cowpea harvesting period in the farmer's field,

i.e., April-June 2024 while the laboratory observation was conducted during July to August 2024.

Procedures

Sampling materials

The survey in the field was carried out using a cluster-random sampling method (Brown and Marshall 1995; Hosang et al. 2015). In each district, five to eight cowpea production centers (districts) were selected. Within each selected district, 10 farmers cultivating the cowpea were randomly selected for seed collection and observation. Number of seeds collected from each respondent farmer varied according to the number of genotypes/varieties cultivated. About 300-500 seeds per variety/genotype were collected from each respondent farmer. A total of 126 cowpea accessions were collected from the survey areas. Out of the 126 collected accessions (Table 1), 26 accessions were collected in Kupang District (accession numbers: KPT, FTL, AFS, OTL, TBN, AMS), 33 accessions in Timor Tengah Selatan District (accession numbers: KTS, ANB, ANS, MLS, KFT, KIE), and 25 accessions were collected in Timor Tengah Utara District (accession numbers: MMT, MFT, BKT, BKS, BTP, BBU, ISN, KFM). Furthermore, several cowpea accessions collected in Belu and Malaka Districts, were, respectively, 18 accessions (accession numbers: RMN, TSB, ATS, RHT, LMN, LMS) and 24 accessions (accession numbers: BTN, LNM, FTB, BKN). Samples of collected cowpea seeds are presented in Figure 1.

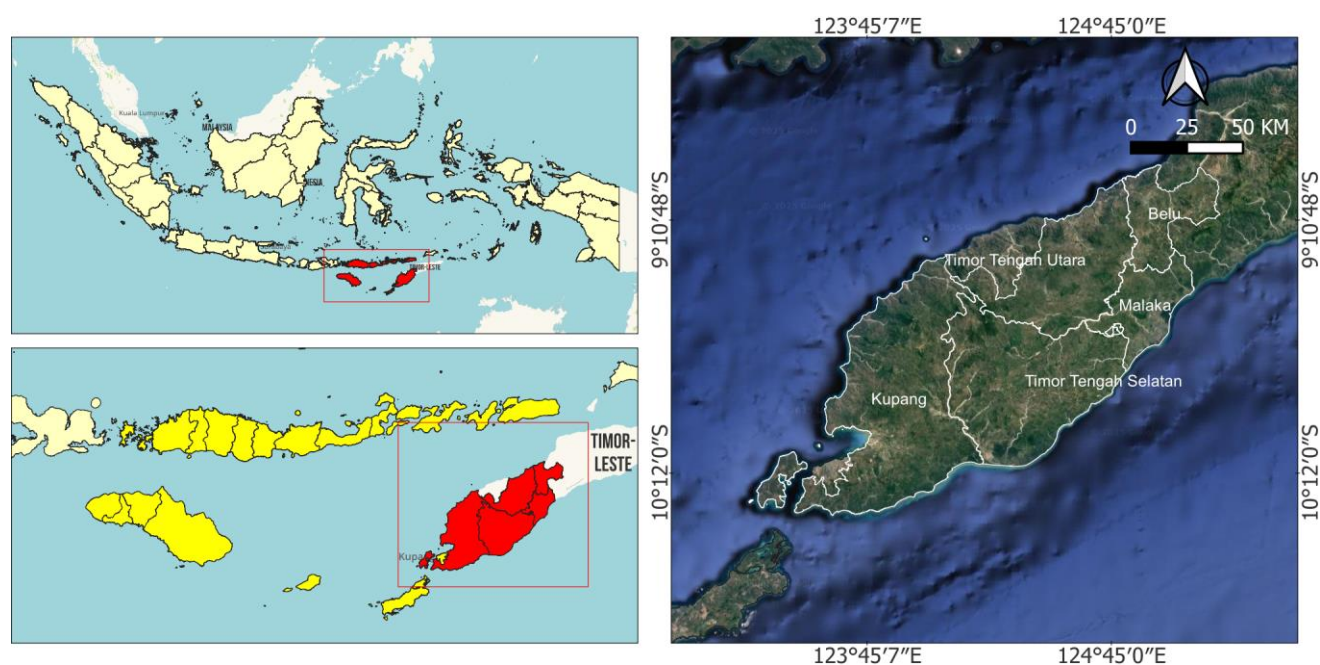


Figure 1. Map of study sites in several districts of West Timor area, East Nusa Tenggara Province, Indonesia, i.e., Kupang, Timor Tengah Selatan (TTS), Timor Tengah Utara (TTU), Belu, and Malaka Districts

Table 1. Expression of morphological and quantitative characters of cowpea accessions from ENT Province, Indonesia

Accession	Seed characteristics									Accession	Seed characteristics								
	Visual observation (VG)					Measurement (MS)					Visual observation (VG)					Measurement (MS)			
	1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9
KPT-2	4	1	0	0	0	1	9	6	12	MFT 2	4	3	0	0	0	1	7	5	12
FTL-1	4	3	0	0	0	1	8	7	10	MFT 3	4	11	0	0	0	1	10	6	14
FTL-2	4	2	0	0	0	1	8	6	8	BKT-1	6	1	0	0	0	1	8	5	10
FTL-3	4	1	0	0	0	1	8	5	12	BKT-2	4	3	0	0	0	1	8	5	12
AFS-1	1	3	0	0	0	1	11	6	14	BKT-3	4	11	0	0	0	3	10	5	16
AFS-2	4	4	1	4	4	1	8	6	12	BKS-1	6	1	0	0	0	1	6	3	14
AFS-3	4	8	0	0	0	1	10	7	14	BKS-2	6	10	1	4	4	1	7	4	14
AFS-4	4	1	0	0	0	1	9	6	12	BKS-3	4	11	0	0	0	1	7	4	10
OTL-1	4	1	0	0	0	1	9	7	12	BKS 1	6	2	1	1	1	1	6	4	8
OTL-2	4	6	0	0	0	1	8	6	12	BTP- 1	6	1	0	0	0	1	9	6	12
OTL-3	4	3	0	0	0	1	8	5	12	BTP -2	6	3	0	0	0	1	8	6	10
TBN-1	4	7	1	2	1	1	7	5	10	BTP-3	6	12	1	1	1	1	10	8	15
TBN-2	4	3	0	0	0	1	8	6	16	BBU-1	4	1	0	0	0	1	8	5	10
TBN-3	4	4	0	0	0	1	6	6	9	ISN-1	4	1	0	0	0	1	10	6	14
TBN-4	4	1	0	0	0	1	8	6	12	ISN-2	4	3	0	0	0	1	7	5	12
AMS-1	4	1	0	0	0	1	6	5	10	ISN-3	6	2	1	1	1	1	7	4	8
AMS-2	4	3	0	0	0	1	7	5	14	KFM-1	4	1	0	0	0	1	7	5	10
AMS-3	4	9	0	0	0	1	5	5	12	KFM-2	4	10	1	4	4	1	8	5	16
AMS-3.1	6	2	0	0	0	1	9	5	12	KFM-3	4	1	0	0	0	1	7	4	12
AMS-1	4	1	0	0	0	1	6	4	8	KFM-4	4	11	0	0	0	1	8	6	14
AMS-2	4	3	0	0	0	1	8	5	14	KFM-5	4	3	0	0	0	1	8	6	16
AMS-3	4	2	0	0	0	1	7	5	10	KFM-6	6	1	0	0	0	1	10	5	12
AMS-4	6	9	0	0	0	1	7	5	14	RMN-1	4	3	0	0	0	1	10	7	14
AMS-4.1	4	8	0	0	0	1	8	5	14	RMN-2	4	1	0	0	0	1	10	8	14
AMS-5	4	1	0	0	0	1	6	5	8	RMN-3	4	5	0	0	0	2	10	6	10
AMS-5.1	6	1	0	0	0	1	6	5	10	RMN-4	4	3	0	0	0	1	9	6	10
KTS -1	4	3	0	0	0	1	8	6	14	RMN-4.1	1	1	0	0	0	1	11	7	16
KTS-1	4	9	0	0	0	1	10	6	13	TSB-1	1	11	0	0	0	1	10	6	12
KTS-2	4	10	1	4	4	1	10	7	17	TSB-2	6	1	0	0	0	1	8	7	8
KTS-3	4	1	0	0	0	1	9	6	12	ATS-1	6	3	1	3	2	1	10	7	16
KTS-4	2	8	0	0	0	1	9	6	11	ATS-2	5	1	0	0	0	1	9	7	12
KTS-5	6	5	0	0	0	1	7	5	11	ATS-3	4	1	0	0	0	1	8	5	10
KTS-5.1	4	9	0	0	0	1	8	5	16	RHT-1	1	1	0	0	0	1	10	7	16
KTS-5.2	4	10	1	4	4	1	10	7	13	RHT-2	4	11	0	0	0	1	10	7	16
KTS-5.3	4	1	0	0	0	1	8	6	11	RHT-3	2	2	1	1	1	1	6	4	10
KTS-6	4	1	0	0	0	1	9	6	11	LMN-1	5	4	0	0	0	1	10	8	14
KTS-6.1	4	3	0	0	0	1	8	5	11	LMN-2	4	1	0	0	0	1	9	6	10
KTS-6.2	4	5	0	0	0	1	9	7	13	LMN 3	6	1	0	0	0	1	9	6	12
KTS-6.3	4	4	0	0	0	1	8	6	12	LMN-4	5	4	0	0	0	1	10	8	14
KTS-7	4	8	0	0	0	1	7	5	11	LMN-4.1	4	1	0	0	0	1	9	6	14
KTS-7.1	4	10	1	4	4	1	8	5	10	LMS-1	6	1	0	0	0	1	10	7	12
KTS-7.2	4	1	0	0	0	1	6	5	9	BTN-1	6	1	0	0	0	1	7	4	12
KTS-7.3	4	2	0	0	0	1	7	5	11	BTN-2	2	3	0	0	0	1	7	4	12
ANB-1	6	1	0	0	0	1	7	5	12	LNM- 1	4	10	1	4	4	1	10	7	14
ANB-2	6	12	1	4	1	1	8	4	10	LNM -2	4	11	0	0	0	1	10	7	14
ANB-3	4	5	0	0	0	1	6	4	10	FTB-1	6	2	1	1	1	1	6	4	12
ANB-4	4	3	0	0	0	1	9	7	10	FTB-2	4	3	0	0	0	1	5	4	12
ANB-5	1	4	0	0	0	1	8	6	12	FTB-3	4	1	0	0	0	1	7	5	8
ANS 1	4	10	1	4	4	1	10	8	18	BKN-1	4	1	0	0	0	1	7	5	10
ANS 2	4	8	0	0	0	1	10	6	16	BKN -1.2	2	3	0	0	0	1	9	5	16
MLS-1	5	8	0	0	0	1	6	6	20	BKN 1.3	4	2	1	1	1	1	7	4	10
MLS-2	4	1	0	0	0	1	7	4	10	BKN -2	2	2	0	0	0	1	8	5	12
KFT-1	4	3	0	0	0	1	6	5	10	BKN-3	4	3	0	0	0	1	7	4	12
KFT-2	4	8	0	0	0	1	6	5	12	BKN -3.2	4	11	0	0	0	1	5	4	12
KFT-3	4	4	0	0	0	1	7	4	12	BKN -4.1	4	10	1	3	4	1	9	6	12
KIE-1	4	4	0	0	0	1	9	6	10	BKN -4.2	1	11	0	0	0	1	10	5	17
KIE-2	4	3	0	0	0	1	10	6	13	BKN -5	6	1	0	0	0	1	8	5	10
KIE-3	6	2	0	0	0	1	8	6	12	BKN-6	4	1	0	0	0	1	9	6	12
KIE-4	4	1	0	0	0	1	8	6	10	BKN-7	1	3	0	0	0	1	10	5	18
MMT-1	4	1	0	0	0	1	10	6	16	BKN -8.1	4	1	0	0	0	1	10	5	12
MMT-2	1	8	0	0	0	3	10	6	16	BKN -9	4	2	1	1	1	1	7	4	12
MMT-3	4	3	0	0	0	1	7	5	10	BKN -9.1	6	3	0	0	0	1	8	5	12
MFT 1	4	1	0	0	0	1	8	5	14	BKN-10	4	4	0	0	0	1	9	6	14

Notes: *Based on cowpea descriptor (IBPGR 1983; PPVTPP 2021). VG: Visual assessment by visually observing 20 seed samples (for characters 1-7), MS: Measurement, by measuring 20 seed samples for seed length and seed width, and weighting 100 seeds for 100-seed weight (for characters 8-9)

Table 2. Seed morphological and quantitative characteristics/ descriptors observed in the present study

Characteristics	Expression and score (phenotypic class)
Seed shape (VG)	1-elliptic; 2-kidney shaped; 3-curved; 4-square; 5-crowder; 6-globose
Seed main color (VG)	1-black, 2-white; 3-red; 4-brown; 5-yellow; 6-cream; 7-whitish red; 8 – reddish brown; 9-maroon; 10-striped brown; 11-light brown; 12-striped red
Presence of secondary color (VG)	0-absent; 1-present
Seed secondary color (VG)	0-absent; 1-black, 2-white; 3-red; 4-brown
Pattern of seed secondary color (VG)	0-absent; 1-around hilum; 2-stripes; 3-partly mottled; 4-fully mottled
Eye color (VG)	1-white; 2-brown; 3-light brown
Seed length (MS) (mm)	measured
Seed width (MS) (mm)	measured
100-seed weight (MS) (g)	weighted

Notes: *Based on cowpea descriptor (IBPGR 1983; PPVTPP 2021). VG: Visual assessment by observing a single plant or a group of plants or parts of plants, MS: Measurement, by measuring several individual plants or parts of plants

Characterization of collected seeds

Sampled/collected seeds were brought to the laboratory for characterization of both morphological and agronomical seed characters. The collected cowpea seeds were characterized using all the seed characteristics provided in the cowpea seed descriptor (IBPGR 1983; PPVTPP 2021). The seed characteristics observed are listed in Table 2. Seed morphological characteristics, such as seed shape, seed main color, presence/absence of secondary color, seed secondary color, pattern of seed secondary color, and eye color were visually observed, and the images of the seeds were qualitatively compared with the standard/descriptor. The measurement of quantitative characters was done on seed length (mm), seed width (mm), and 100-seed weight (g). Seed length and seed width were measured using a digital caliper measuring the longest and widest parts of the seeds while 100-seed weight was weighted using a digital scale.

Data analysis

The obtained qualitative data were descriptively analyzed and presented in the form of tables and pictures. The visually observed qualitative seed characters were given scores based on their phenotypic expression (IBPGR 1983; PPVTPP 2021) prior to statistical analysis. Both the scores of qualitative and quantitative characters were subjected to cluster analysis based on Euclidean distance and un-weighted pair group method with arithmetic mean (UPGMA) to construct a dendrogram and determine the genetic diversity of the cowpea germplasm (Mafakheri et al. 2017; Nkhoma et al. 2020). Further, a Principal Component Analysis (PCA) was also conducted to determine the characters that mostly contributed to the total observed variation in the data set. The statistical analysis was conducted using PAST version 4.03 (Hammer et al. 2001).

RESULTS AND DISCUSSION

Observed qualitative and quantitative seed characters

Samples of collected cowpea seeds are presented in Figure 2, while the observed phenotypic class of seed

characters are presented in Table 1. The results showed that only one character (16.67%), i.e., the presence of secondary color, out of the six observed qualitative/morphological characters was dimorphic, while the remaining five characters (seed shape, seed main color, seed secondary color, pattern of seed secondary color, eye color) (83.33%) were polymorphic (≥ 3 expressed phenotypic classes), and none was monomorphic for all observed cowpea accessions (Table 1). The dimorphic character was the presence/absence of seed secondary color (no. 3) while the polymorphic characters included seed shape, seed main color, seed secondary color, pattern of seed secondary color, and eye color. Similarly, the seed quantitative characters also varied among the cowpea accessions with seed length range of 5-11 mm, seed width range of 3-8 mm, and 100-seed weight range of 8-20 g.

The distributions of seed morphological phenotypic classes and quantitative characters are presented in Table 3. Five phenotypic classes were observed on the seed shape, i.e., elliptic, kidney-shaped, square, crowder, and globes. Of the 126 collected accessions, 85 accessions (67.46%) possessed a square seed shape, followed by the globose (19.05%), while the rest of the seed shape classes ranged from 3 to 6% of the total collected accessions. The collected cowpea germplasm varied considerably in the seed main color with a total of 12 phenotypic classes (Table 1), of which black is the dominant seed color (33.33%) followed by red (20.63%) and white (9.52%) at the second and the third places, respectively.

The secondary colors were observed on 19 (15.08%) cowpea accessions while the rest 107 accessions (84.92%) showed no secondary color (Table 3). Four phenotypic classes of secondary color were observed, i.e., brown (50%), black (35%), red (10%) and white (5%). Of the 19 accessions that exhibited secondary color, 9 accessions had secondary color around the hilum, 9 accessions had fully mottled secondary color, and one accession had stripes secondary color. The majority (97.62%) of the collected cowpea accessions exhibited white eye color, while the rest had light brown (1.50%) and brown (0.79%) eye color.



Figure 2. Samples and source (district) of cowpea germplasm seeds collected in West Timor. A. Without secondary seed color (above, left to right: Belu, TTU, and Malaka Districts; below, left to right: Malaka, TTU and Kupang Districts); B. With secondary seed color (above, left to right: TTS and Kupang Districts; below, left to right: Malaka and TTS Malaka Districts)

Genetic diversity

The observed qualitative seed character scores were dimorphic and polymorphic, while the quantitative seed characters varied considerably among the germplasm with a seed length range of 5-11 mm, seed width range of 3- 8 mm while that of the 100-seed weight was 8-18 g. Thus, both qualitative and quantitative data were used to determine the genetic diversity of the collected cowpea germplasm. The resulting dendrogram showing the clustering of the cowpea germplasm is presented in Figure 3. The dendrogram showed that 126 cowpea accessions are grouped into three clusters (I, II, and III) at a truncation point of 8.0. Cluster I only consist of one accession (MLS-1), Cluster II consists of two subclusters, and Cluster III also consists of two subclusters. Cluster II comprises 31 accessions, 22 accessions in subcluster 1 dan nine accessions in subcluster 2. Cluster III comprises 94 accessions, the majority of which were in subcluster 1 (92 accessions) and only two accessions comprised subcluster 2. In addition, eight duplicates (accessions that are grouped at 0.0 truncation point) are present in Cluster III, indicating that the accessions constituting each duplicate are more likely the same genotype (Figure 3).

Principal Component Analysis (PCA) revealed that the total observed variation in the data set was contributed by 9 independent components (principal components, PC). The first four principal components (PC) with eigenvalues >0.8 were responsible for 81.70% of the total observed variation. The first four components included PC1 (34.74%), PC2 (24.73%), PC3 (12.95%), and PC4 (9.23%). The observed seed characters used in the PCA analysis revealed biplots of PCA (PC1 vs. PC2, PC1 vs. PC3, and PC1 vs. PC4) (Figure 4). The results of principal component analysis revealed four seed morphological characters with positive and the highest loading factors (0.34-0.53) that were responsible for the maximum

variability in principal component 1 (PC1), which explains 34.74% of the total observed variability in the data set. The four most contributing characteristics included seed color, the presence/absence of secondary seed color, secondary seed color, and pattern of secondary seed color. Meanwhile, the seed characters (with the highest and positive loading scores of 0.43-0.54) mostly contributing to maximum variability (24.73%) in principal component 2 (PC2) were seed length, seed with and 100-seed weight (Figure 4.A).

The observed variability in principal component 3 (PC3) (12.95%) was mostly contributed by two characters with positive and the highest loading factors, i.e., seed shape (0.5) and seed width (0.4) (Figure 4.B). The seed characters mostly responsible for maximum variability (9.23%) in component 4 (PC4) were seed shape and seed eye color with loading scores of, respectively, 0.72 and 0.66 (Figure 4.C).

Discussion

The collected cowpea germplasm varied considerably in seed coat color with 12 phenotypic classes (Table 3). In comparison, Nkhoma et al. (2020) examined 90 cowpea accessions from Southern Africa, and they found only five phenotypic classes of seed coat color, i.e., brown (49 accessions), black (21 accessions), purple-brown (12 accessions), white (10 accessions) and red-brown (8 accessions). Additionally, Tyagi et al. (2024) examined the seed coat color of 25 cowpea genotypes from India, and found 19 genotypes as pigmented (black, brown, dark brown, grey, dark grey seed color) and six genotypes as unpigmented (cream and white seed color). Thus, in terms of seed color, the cowpea germplasm examined in this study had very high genetic diversity, which allows a more flexible selection of parents with certain seed coat colors for cultivar development. The diversity of the cowpea seed

color is derived from a complex network involving genetic and environmental interactions (Boukar et al. 2019; Lonardi et al. 2019). Seed coat pattern and color in cowpea are controlled by many genes (quantitative trait loci) suggesting a complex genetic interaction (Herniter et al. 2019; Ajayi et al. 2020; Xiong et al. 2024).

The present study showed black as the dominant (33.33%) seed coat color of the collected cowpea accessions, indicating the farmer's preference for black seed coat color. As a comparison, a study on the diversity of cowpea seed color by Yasin et al. (2021) revealed that cream-brown seed color was dominant, followed by white, black, and brown. Nkhoma et al. (2020) stated that cowpea qualitative characteristics, such as seed coat color, pod color, and leaf color affect the market value of cowpea in Africa since the farmer and consumer preferences are based on these traits. Furthermore, Boukar et al. (2019) highlighted the association between the cowpea seed coat color and processing quality, more specifically, the cooking time, and farmers deliberately select varieties that have shorter cooking times. Thus, breeding programs need to focus on developing cowpea varieties that meet the consumer's preferences and market requirements. Herniter et al. (2018) found that the inheritance of black seed coat color is controlled by major genes, which allow easier and more effective selection in cowpea cultivar development. Further, Ajayi et al. (2020) reported that seed coat color in cowpea was polygenically controlled, and the black color was dominant over brown color. Major genes, with codominant gene action, controlling the black seed coat color had been reported in mungbean (Mau et al. 2023).

Six phenotypic classes of seed shape were encountered in the present study (Table 1), i.e., elliptic, kidney-shaped, curved, square, and crowder, with square seed shape being the most dominant (67.46%). As a comparison, Makanur et al. (2012) obtained only three phenotypic classes of seed shape of 35 cowpea genotypes examined, i.e., ovoid (three), kidney-shaped (seven) and remaining as rhomboid. Further, Yasin et al. (2021) observed five seed shapes of 36 cowpea accessions from Southern Ethiopia, the majority (33%) of which were rhomboid. Assessment of seed character diversity among cowpea accessions is important for both germplasm conservation and cultivar development of cowpea. The use of seed characters/traits for the assessment of genetic diversity in cowpeas has been previously reported by Nkhoma et al. (2020) and Widajati et al. (2023). The genetic diversity of crops has frequently been assessed using both cluster analysis and principal component analysis (PCA) (Venujayan et al. 2017; Mau et al. 2017, 2022; Ochieng et al. 2019; Seid et al. 2021).

The cluster analysis grouped the accessions into three main clusters. Cluster I was composed of only one accession while each of Cluster II and Cluster III was further subdivided into two subclusters. Accession MSL-1 constituted stand-alone Cluster I and is distinguishable from other accessions due to its unique higher 100-seed weight (20 g), indicating its larger seed size as compared to that of other accessions. The 100-seed weight is an important seed trait as this attribute may be associated with seed yield and also

the consumer's preference. Previous studies reported that the 100-seed weight of cowpea was highly correlated with seed yield and plant height at maturity (Owusu et al. 2021), but tended to be negatively associated with seed germination, seed vigor index, and seed germination rate (Widajati et al. 2023). 100-seed weight is highly correlated with seed size (Lopes et al. 2003). The seed size of legume crops, including cowpea, is affected by genetic make-up and, in a certain degree, by the environment (Gunathilake et al. 2016). Egbadzor et al. (2013) reported that the seed size of cowpea genotypes grown in Ghana is controlled by eight genes, and the small seed size was partially dominant over the large seed size.

Table 3. Distribution of seed qualitative and quantitative data of cowpea accessions from ENT Province, Indonesia

Qualitative characteristics			
Seed characters	Expression (phenotypic class)	Number of accessions	Percentage (%)
Seed shape	1-elliptic	8	6.35
	2-kidney shaped	5	3.97
	3-curved	0	0.00
	4-square	85	67.46
	5-crowder	4	3.17
	6-globose	24	19.05
Seed main color	1-black	42	33.33
	2-white	12	9.52
	3-red	26	20.63
	4-brown	9	7.14
	5-yellow	4	3.17
	6-cream	1	0.79
	7-whitish red	1	0.79
	8-reddish brown	8	6.35
	9-maroon	4	3.17
	10-striped brown	8	6.35
	11-light brown	9	7.14
	12-striped red	2	1.59
The presence of a secondary color	0-absent	107	84.92
	1-present	19	15.08
Seed secondary color	1-black	7	35.00
	2-white	1	5.00
	3-red	2	10.00
	4-brown	10	50.00
The pattern of the seed's secondary color	1-around hilum	9	47.37
	2-stripes	1	5.26
	3-partly mottled	0	0.00
	4-fully mottled	9	47.37
Eye color	1-white	123	97.62
	2-brown	1	0.79
	3-light brown	2	1.59
Quantitative characteristics			
Seed character	Expression (unit)	Range	
Seed length	mm	5-11	
Seed width	mm	3-8	
100-weight	g	8-20	

Note: * Each data point was obtained from the observation of 20 seeds per accession

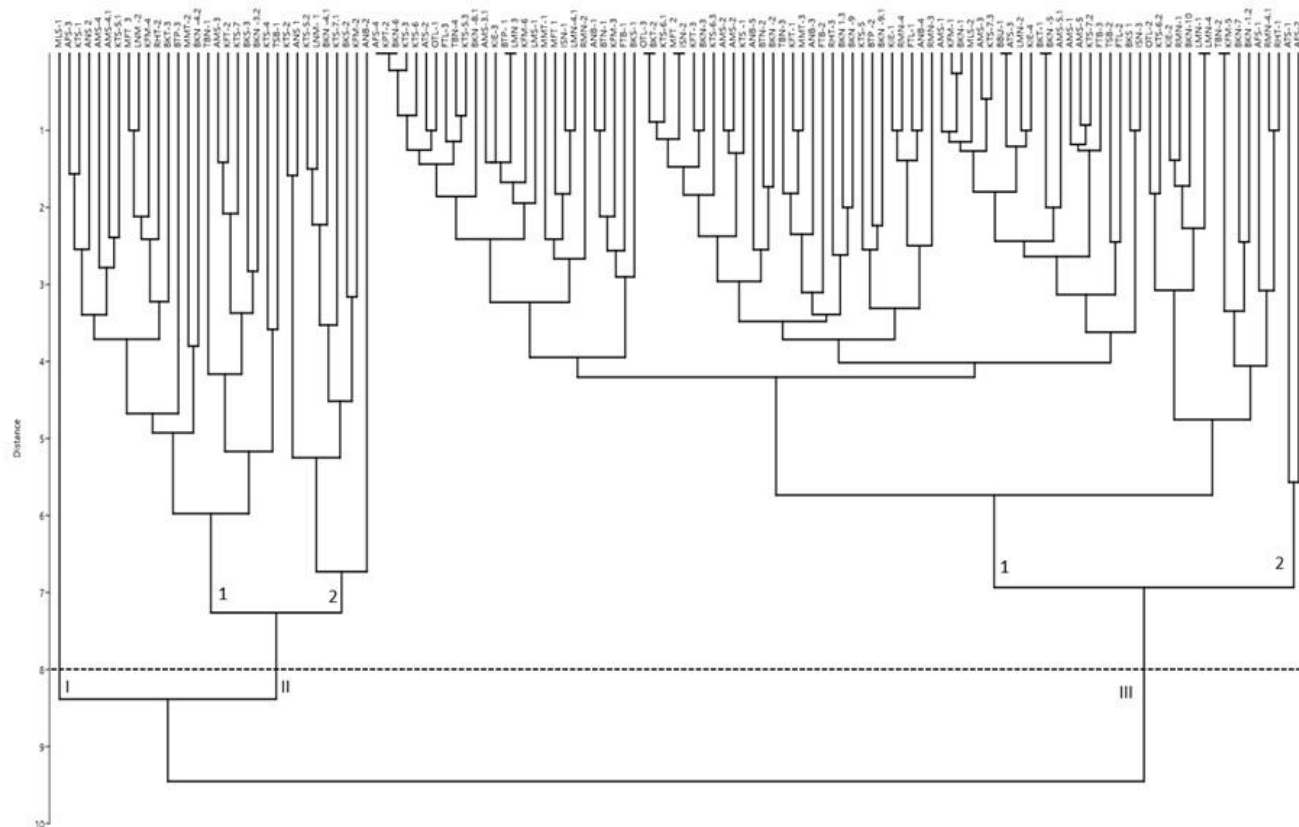
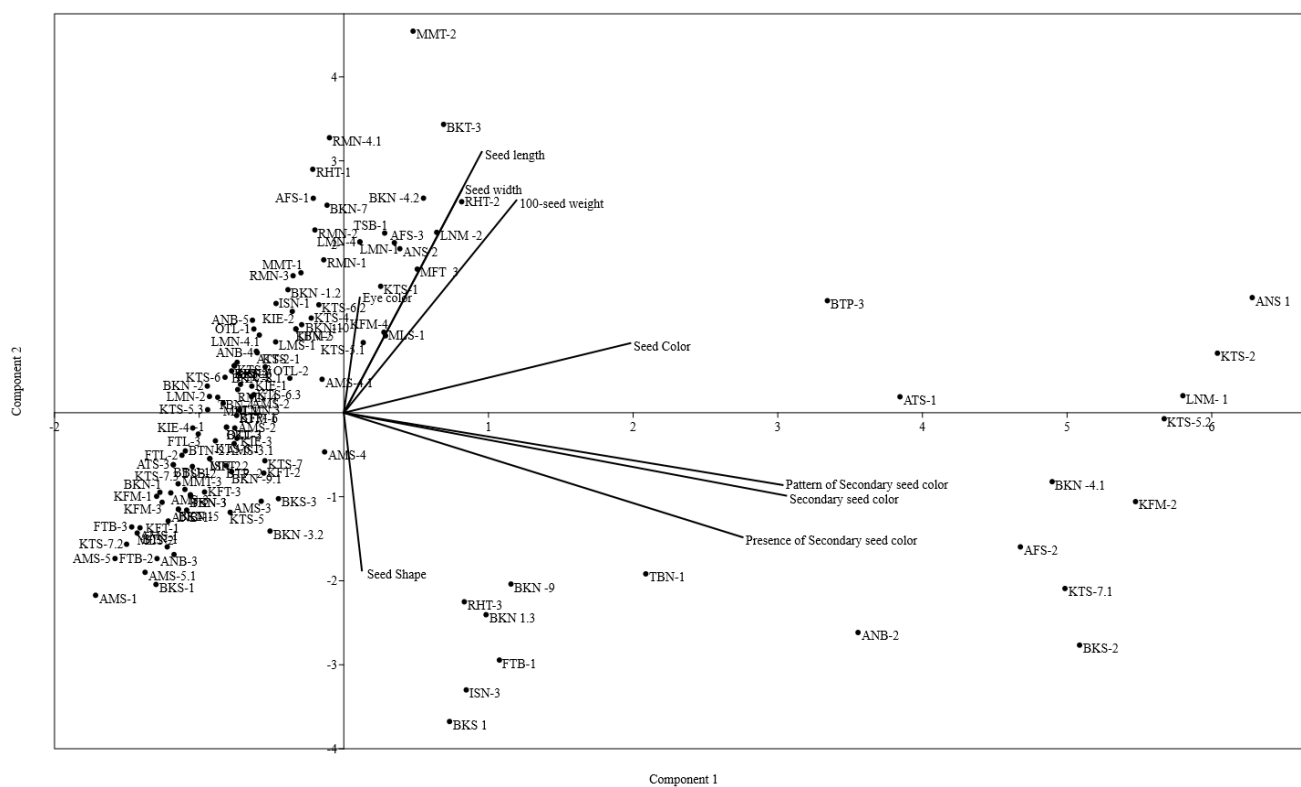


Figure 3. A dendrogram among 126 cowpea accessions using Euclidean distance coefficient based on nine seed characters



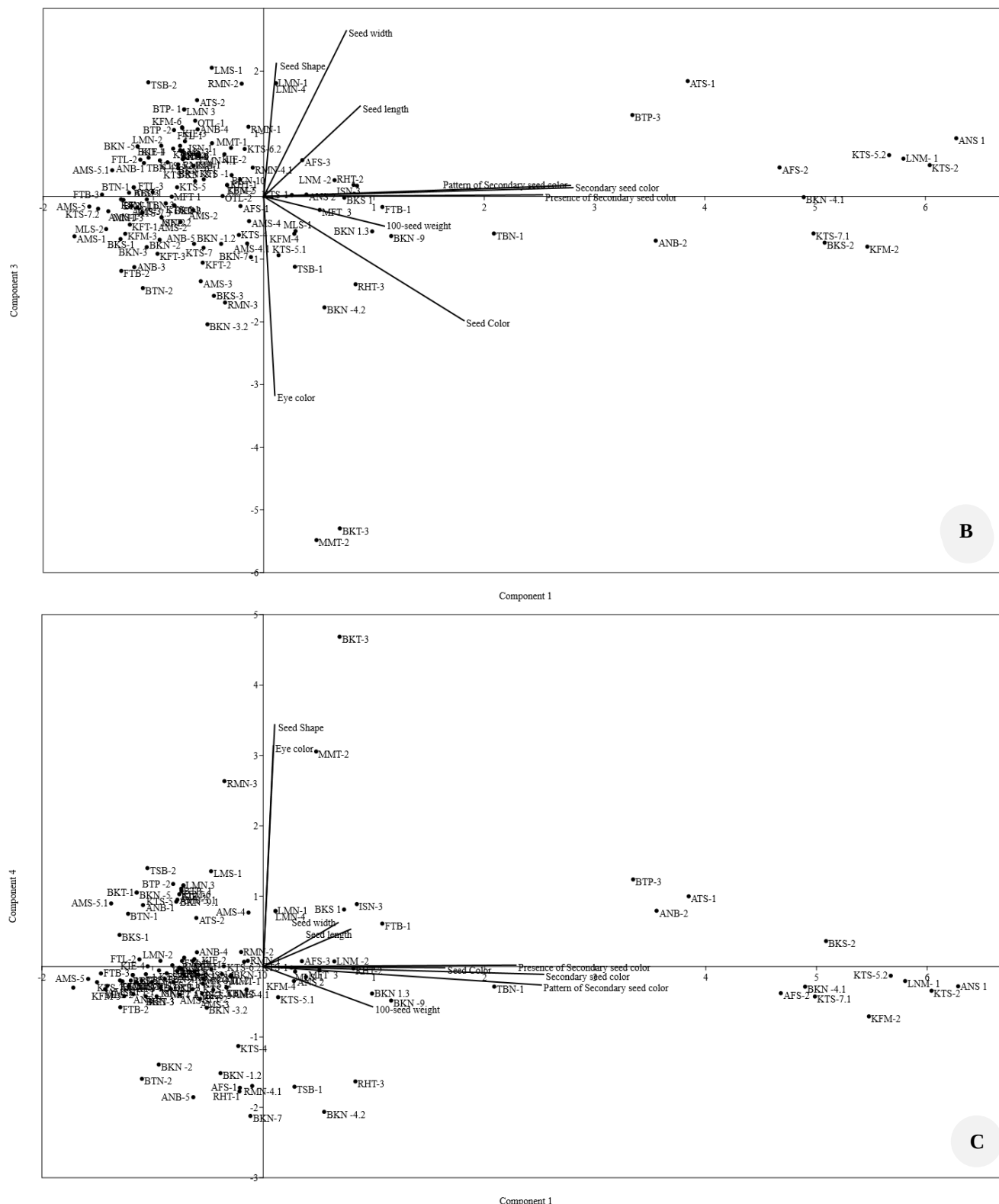


Figure 4. Scatter plots showing the distribution of the examined cowpea germplasm and seed characters mostly responsible for the total variability observed in the 126 cowpea accessions in A. PC1 versus PC2; B. PC1 versus PC3; C. PC1 versus PC4

The clustering of the studied accessions into several clusters and sub-clusters indicates that seed morphological and quantitative characteristics used in this study effectively

revealed the genetic diversity of the cowpea germplasm. The present study results are in line with Widajati et al. (2023) who examined 10 cowpea genotypes from the Plant

Breeding Laboratory of IPB and the Indonesian Research Institute for Legumes collections and concluded high variability among the tested genotypes based on seed morpho-physiological characters and seed viability. The tested genotypes were also grouped into three major clusters on the observed characters. In addition, Widajati et al. (2023) also found that the tested genotypes possessed superior seed morpho-physiological and viability. Thus, they can further be employed for superior cowpea variety development. The effectiveness of seed characters for discrimination of cowpea genotypes has been reported by several authors, such as Nkhoma et al. (2020), Yasin et al. (2021), and Mofokeng et al. (2021).

The study revealed nine principal components (PC) that were responsible for the total observed variation in the data set, the first four of which were responsible for 81.70% of the total observed variation. Of the four components, seed characteristics that were mostly responsible for the observed variability included seed color, secondary seed color, seed shape, seed length, seed width, and 100-seed weight. The use of PCA in cowpea genetic diversity analysis was previously reported by Reddy et al. (2023), who found six principal components that contributed 95.43% variability by nine quantitative characters studied, of which maximum contribution was obtained from characters such as pod number, peduncle number, primary branches, and seed yield. Furthermore, Lazaridi et al. (2017) reported that principal component analysis classified the majority of local cowpea populations in Greece into two clusters (mainly based on seed coat color and eye color). The use of PCA in cowpea genetic diversity analysis has also been reported by Osho and Olasanmi (2019), in which, observed variability was maximally contributed by traits such as growth pattern, plant pigmentation, plant hairiness, pod attachment to the peduncle, leaf color, immature pod pigmentation, twinning tendency, terminal leaflet shape and pod color.

The present study revealed that the dendrogram did not correspond with the geographical area of the seed collection sites but rather with the seed characteristics. The clustering of the cowpea genotypes corresponds with that of the PCA biplot, where the genotypes sharing similar characteristics were grouped in the same cluster/sub clusters or the same biplot quadrant. The present results are in line with Osho and Olasanmi (2019), who concluded that PCA, in combination with other analyses such as biplot and cluster analysis, was efficient in the genetic diversity analysis of cowpeas for selection purposes.

In conclusion, the results showed high genetic diversity among the accessions based on seed qualitative and quantitative characteristics. The cluster analysis results revealed that the collected accessions were grouped into three clusters, which corresponded with seed characteristics. The PCA revealed four main principal components (PC) that explained most (81.70%) of the observed variability in the data set. The study results will be useful the germplasm conservation as well as its utilization in plant breeding programs for the development of superior cowpea varieties.

ACKNOWLEDGEMENTS

We are very thankful to the Rector of Universitas Nusa Cendana, Kupang, Indonesia, for supporting this research through the *Penelitian Dasar Unggulan Perguruan Tinggi* (PDPT) Research scheme in fiscal year 2024.

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