

Morphological characteristics and variability of traditional starch forming coix (*Coix lacryma-jobi* var. *ma-yuen*) populations from Mindanao Island, Philippines

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Abstract. Bon SG, Antesco GP, Enicola EE. 2023. Morphological characteristics and variability of traditional starch forming coix (*Coix lacryma-jobi* var. *ma-yuen* populations from Mindanao Island, Philippines. *Biodiversitas* 24: 4382-4391. *Adlay* (*Coix lacryma-jobi* var. *ma-yuen* (Rom.Caill.) Stapf) is an underutilized crop in the Philippines with high potential. Characterization and elucidation of the available variability among the local *adlay* populations are essential information for its improvement and better utilization. The study aimed to characterize the qualitative morphological traits of 34 traditional *adlay* populations from Mindanao Provinces and assess diversity by the Shannon Index and similarity by multivariate clustering. Results showed that all populations shared the same morphotypes in five descriptors but were variable for the rest. Six populations showed intense leaf anthocyanin coloration, and 23 expressed upright growth habits. Three types of leaf blade colors were observed, while the intensity of anthocyanin coloration of culms was expressed in four types. Most populations had semi-pubescent or highly pubescent leaves, generally intermediate leaf orientation and non-glaucous leaves. Immature grains were generally light green but expressed four color types at maturity, mostly circular with furrows on the surface. Diversity Indices (*H'*) ranged from low to high. Leaf blade pubescence, anthocyanin coloration of culm, and mature grain color had high *H'* values. Cluster analysis showed that the variability was narrow, where overall clustering achieved 0.50 similarity. At 0.65 similarity, three groups can be derived with three populations as outliers. Clustering can be attributed to the differences in seedling color, anthocyanin coloration, plant growth habit, immature grain color, mature grain color, and shape. The study established the morphological description of the qualitative traits of traditional Philippine *adlay* and assessed diversity to be generally intermediate, where populations can be clustered into subgroups according to the degree of morphotype similarities. Clustering, however, was not related to provenances. It is recommended to conduct further geographic germplasm sampling, marker-based characterization, and agronomic evaluation to dissect further the genetic diversity and crop potential of the local *adlay* populations.

Keywords: *Adlay*, cluster analysis, coix, diversity, morphological diversity, Philippine *adlay*, Shannon Diversity Index

INTRODUCTION

Adlay (*Coix lacryma-jobi* var. *ma-yuen* (Rom.Caill.) Stapf), the starch-forming coix, is a minor cereal crop grown in East, South, and Southeast Asia. In the Philippines, indigenous communities of Bukidnon, Cotabato, and Zamboanga Peninsula Provinces in Mindanao Island have been known to grow and consume *adlay* (Wester 1922). It is cultivated as a secondary subsistence crop by the *Subanen* Tribe in Zamboanga del Sur District (BDP 2022) and by the *Manobo*, *Bagobo*, and *Talaandig* Tribes in Mindanao's mountainous regions. It is grown in areas where rice and corn do not grow well (Slow Food 2019). Some popular farmers varieties are *Gulian*, *Guinampay*, *Pulot/Tapol*, and *Linay* (BM 2022). *Adlay*, however, remains a traditional subsistence crop; hence, the extent of cultivation and production information is wanting. However, some local governments included *adlay* in their agricultural initiatives, like Bukidnon, reported around 500 hectares planted to *adlay* (RadyoMan-Manila 2020).

Adlay contains about 67.2% starch, 14.2% protein, and 3.6% fat (Corke et al. 2016), 0.8g fiber, 1.9g ash, 25mg Ca,

435mg P, 5.0µg Fe, 0.28mg thiamine, 0.19mg riboflavin, 4.3mg niacin for every 100g grains (Liu et al. 2019). It is prepared in traditional homemade recipes brewed into tea or fermented alcoholic drinks. It can also be roasted as a coffee substitute (Arora 1977; Corke et al. 2016). In the Philippines, *adlay* is consumed as steamed cereal, fermented alcoholic drink, and in a variety of generally sweetened snack foods like *Biko*, *Suman*, or *Kutsinta* among the indigenous communities (Slow Food 2019). In parts of South and East Asia, it has been used historically in folk medicines to remedy numerous common ailments and for general nourishment (Yu et al. 2017; Liu et al. 2019). Recently, its potential use in modern medicine to address a variety of lifestyle, degenerative, auto-immune conditions, tumors, and cancers (Kuo et al. 2012; Yu et al. 2017) and as functional food (Zeng et al. 2022) is gaining attention.

Adlay is a grass species in the tribe Maydeae and is closely related to sorghum and maize within the cereal species (Liu et al. 2019). It is monoecious open-pollinated species (Feng et al. 2020) forming female-sterile and female fertile spikelets where the pollen-bearing male flower is borne on a slender stalk protruding out of the

spikelet through a small opening along with stigma. Starch-forming *adlay* is diploid with karyotype designation, $2n=20$ (Corke et al. 2016). While various mountain tribes in Southeast Asia cultivate it, its historical records and germplasm diversity are predominantly found in Southwest China, including Northeast India and Burma (Arora 1977; Weng et al. 2022). Records of *adlay* date back to about 8000 years ago in China (Weng et al. 2022) and 4000 years ago in India, where 23 local names were recorded (Kumar et al. 2014). Merrill (1912) mentioned its probable prehistoric introduction in the Philippines and listed 32 local names. Wester (1922) reported that tribes in Mindanao called it "Adl'y," which eventually became known as *adlay* or *adlai* in English. Across South, East, and Southeast Asia, indigenous *adlay* populations exhibit a wide range of diversity. In India, *adlay* can be classified based on involucre size, color, and texture (Arora 1977). Considering grain characteristics, Shen et al. (2019) observed variability among Chinese *adlay* populations. China is home to five species of coix (Weng et al. 2022). Similarly, Wester (1922) noted variability among Philippine *adlay* regarding grain shape and size. The initial establishment of the basic variability profile in plant germplasm often involves morphological characterization and assessment of variability using standard statistical estimates and procedures. Phenotypic characterization of most crops is typically carried out using the crop's standardized descriptors lists when available. Using statistical tools such as Diversity Indices and multivariate analyses, observations can provide insights into the nature and pattern of variation within a germplasm set. Therefore, this study aimed to describe the qualitative morphological characteristics, estimate the proportion of observed variability and assess the level of differences and similarities among 34 populations of cultivated starch-forming *adlay* from the Mindanao region in the Philippines. Findings are expected to contribute to the information and understanding of the characteristics and nature of diversity of Philippine *adlay* for effective crop varietal development efforts for improved *adlay* cultivation.

MATERIALS AND METHODS

Adlay germplasm populations

Local traditional *adlay* composed of thirty-four (34) populations collected from the Mindanao Provinces, Philippines namely, Bukidnon (24), Zamboanga del Sur (3), Agusan del Sur (1), South Cotabato (1), Saranggani (1), Misamis Occidental (1), Lanao del Sur (3) were used in the study. Materials were obtained from the National Plant Genetic Resources Laboratory (NPGRL) of the Institute of Plant Breeding of the College of Agriculture and Food Science of the University of the Philippines Los Banos, Philippines. All *adlay* germplasm populations were cultivated starch-forming traditional farmers' variety types.

Morphological observation

Study populations were planted in the field plots set up for morpho-agronomic characterization in systematic plot

arrangement following the open-pollinated procedures for corn germplasm. Land preparation followed the general procedure of corn and sorghum germplasm field setup. Grow-out was set up in a systematic plot arrangement where each entry was planted in six rows of 5m. Thus, a plot consisted of 6 plant rows distanced 1m apart while plants (hills) within the row were 0.70m apart. Pre-germinated seeds are sown directly. Irrigation and crop management practices followed the general recommendation for open-pollinated cereal germplasm followed at the Institute of Plant Breeding (IPB). Crop protection measures were applied as needed. Field entries were observed for twenty qualitative morphological traits, namely, seedling color, the intensity of anthocyanin coloration, plant growth habit, leaf pubescence, the shape of apex, the color of the leaf blade, leaf sheath pubescence, leaf orientation, ligule presence, culm color, intensity of anthocyanin coloration of culm, culm glaucosity, anther color, stigma color, color of immature grains, panicle branching, color of grain at maturity, grain shape, presence of furrows on grains. Plant trait observation and data gathering were done at the stages of the seedling, maximum vegetative, flowering, immature grain, and dry mature grain. Traits observed were adopted from the Standard Descriptors List for Corn (IBPGR 1991) and the UPOV (2012) Guidelines for the Conduct of Test for Distinctness, Uniformity, and Stability for *Adlay*. The observation was based on the general appearance of the plants within the plot. The observation was based on the general appearance of the plants within the plot.

Data analyses

Estimate of diversity by Shannon Diversity Index

Raw data were encoded and processed in the Microsoft Excel® spreadsheet application as a rectangular data input matrix for the statistical procedures. Levels of genetic diversity were assessed using the Shannon Diversity Index (SDI) calculated by the following formula:

$$H' = - \sum_{i=1}^n (p_i * \ln p_i)$$

Where:

p_i : Relative frequency of occurrence

$\ln p_i$: Natural logarithm of the relative frequency

n : Number of observed classes or morphotypes

The summation is standardized following the equation:

$$(-H' * -1) / H_{\max}$$

Where:

H_{\max} : $\ln(S)$

S : Number of known classes or morphotypes

Cluster analysis

Multivariate statistical analysis by clustering using the twenty qualitative morphological traits was performed using the commercial desktop application NTSys-pc v.2.21q (Rohlf 2008). The similarity matrix was derived using the similarity/dissimilarity function with a simple

matching coefficient. The 20 multivariate similarity data matrix was used as the input matrix for the clustering procedure. Clustering was performed following the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) and phylogenetic clustering in the Sequential, Agglomerative, Hierarchical Nested (SAHN) methods (Rohlf 2008). The tree plot function visualized the resulting cluster as a graphical dendrogram.

RESULTS AND DISCUSSION

Qualitative morphological description of Mindanao *adlay* populations

A summary of the observed morphotypes for the qualitative morphological traits of the 34 traditional *adlay* populations from Mindanao provinces is shown in Table 1. Of the 20 traits considered, all populations have a pointed leaf apex shape, ligules, green culms, yellow anthers, and purple stigma. A higher frequency of purple stigma color in the *adlay* germplasm collection set was also reported by Cong et al. (2023). For the rest of the morphological traits, the populations expressed variable types. At the seedling stage, color had two types observed with BK2016-10, GB64777BK, GB64782BK, GB64788BK, GB65568AS, BRS2016-23ZS, GB2016-22ZS, and GB60305SC having dark green seedlings while the rest were rated as green. The intensity of anthocyanin coloration at the seedling stage was either weak or strong. Strong anthocyanin intensity was observed on BK2016-10, GB61477BK, GB61478BK, GB61477BK, and GB614782KB. Plant leaf anthocyanins have been associated with growth stress protective responses, particularly high light stress (Zhao et al. 2022). While vegetative anthocyanin coloration was primarily used as a trait descriptor for characterization in this study, the six populations with strong anthocyanin coloration form a potentially important part of the Philippine *adlay* germplasm for work involving such traits.

Plant growth habit was mostly upright (Figure 1.A), with eleven populations being semi-upright (Figure 1.B). Semi-upright populations are BK2016-7, GB64777BK, B64781BK, GB61477BK, GB61523BK, GB64775BK, BK2016-003, GB63370ZS, BRS2016-23ZS, GB2016-22ZS and GB60305SC. Upright growth habit is associated with the modernity of crop varieties. Thus, open and semi-erect culms are generally of traditional germplasm. Chaniago et al. (2022) reported that nearly 70% of Indonesian traditional germplasm studies were of open and semi-erect growth habits in rice. Agronomically, Tafere and Irie (2019) noted in rice that erect culm was associated with better light interception and nitrogen use efficiency, hence better crop productivity. The current *adlay* population set is a potential mixture of germplasm for growth habit types that may be evaluated for varietal improvement of the local *adlay* concerning the agronomic trait. Leaf pubescences observed were smooth, semi-pubescent, or highly pubescent. Most of the populations were either semi-pubescent or highly pubescent. Only GB64782BK and GB2016-22ZS had smooth leaves. Pubescence in crop leaves is considered a physiologically

beneficial characteristic. Hu et al. (2013) cited that leaf trichomes were involved in physiological processes such as photosynthesis, transpiration, respiration, and grain yield formation. The potential agronomic role of leaf trichomes in increasing water use efficiency in crops, particularly in water-limited environments. However, Hu et al. (2013) noted that smooth or glabrous leaves and grains were preferred to handle in crop tending and harvesting as it is less itchy. Being a minor crop, highly pubescent populations can be specifically evaluated for their adaptability to moisture-challenged environments in contrast to their natural adaptation to wet ecosystems. Some studies have noted the role of trichomes in plant water regimes, such as in *Caragana korshinskii* Kom. an arid species (Hu et al. 2013) and *Solanum lycopersicum* L. (Galdon-Armero et al. 2018). The leaf pubescences observed among the present *adlay* populations offer a range of types that may be further evaluated and harnessed for potential utilization in *adlay* crop development.

The populations exhibited three leaf blade colors in the green hue: light green, green, and dark green. The intensity of anthocyanin coloration of culms was expressed in four types: absent to very weak, weak, medium, and strong (Figure 2.A to 2.D). The collection set of Mindanao *adlay* showed a range of expressions in the presence of culm anthocyanin coloration, a characteristic observed at the late vegetative stage. Landi et al. (2015) reviewed in detail the primary putative functions of plant anthocyanin, such as serving as sunscreens and anti-oxidants, mediators of reactive oxygen species-induced signaling cascades, chelating agents for metals or metalloids and delayers of leaf senescence, especially in plants growing under nutrient deficiency. In particular, Liang and He (2018) discussed the substantial role of anthocyanin in plants for tolerance to low nitrogen growing conditions in *Arabidopsis* Heynh. Hence, *adlay* germplasm populations expressing the characteristics may serve as material for investigations on the potential value of the trait in *adlay* growth development. The leaf sheath color was primarily light green, with two populations, BK2016-7 and BK2016-10, being dark green. Leaves were mainly immediately oriented, but BK2017-3 and GB64777BK had erect leaf orientation. Culm glaucosity was present in three populations only: GB64875BK, BRS2016-23ZS, and GB2016-22ZS (Figure 3.A). The rest of the populations did not exhibit the presence of glaucous formation on culms (Figure 3.B). Weng et al. (2022) noted that wax covering on stalk epidermis and flag leaves among Chinese *adlay* germplasm might reduce transpiration during warm periods during water entry during wet periods. Thus, associating the feature with the ability of *adlay* to thrive in drought and water-logged conditions. In wheat and barley, waxes on the leaf, stem, and spike were associated with high-yield performance under drought conditions (Hen-Avivi et al. 2016). Therefore, the three populations with waxy culm may serve as valuable germplasm for local *adlay* varietal development and cultivation research. Immature grains exhibited four color types: light green and purple hues of light purple, purple, and dark purple.

Table 1. Observed morphotypes of traditional *adlay* populations from Mindanao Provinces, Philippines

| Population ID | Province | Seedling color | Intensity of anthocyanin coloration | Plant growth habit | Leaf pubescence | Shape of apex | Leaf blade color | Leaf sheath color | Leaf-sheath pubescence | Leaf orientation | Ligule presence | Culm color | Intensity of anthocyanin coloration of culm | Culm glaucosity | Anther color | Stigma color | Color of immature grains | Panicle branching | Color of grain at maturity | Grain shape | Furrows on grain |
|---------------|--------------------|----------------|-------------------------------------|--------------------|------------------|---------------|------------------|-------------------|------------------------|------------------|-----------------|------------|---|-----------------|--------------|--------------|--------------------------|-------------------|----------------------------|-------------|------------------|
| Bk2017-2 | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Bk2016-5 | Bukidnon | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Bk2016-6 | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Bk2016-7 | Bukidnon | Green | Weak | Semi-upright | Highly pubescent | Pointed | Dark green | Dark green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Bk2016-8 | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Bk2016-10 | Bukidnon | Dark green | Strong | Upright | Highly pubescent | Pointed | Dark green | Dark green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Bk2017-3 | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Highly pubescent | Erect | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Bk2017-5 | Bukidnon | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Medium | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Gb61559bk | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Gb63029bk | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Light brown | Circular | Present |
| Bk2016-003bk | Bukidnon | Green | Weak | Semi-upright | Highly pubescent | Pointed | Green | Light green | Smooth | Intermediate | Present | Green | Medium | Absent | Yellow | Purple | Light green | Present | Dark brown | Circular | Absent |
| Gb61477bk | Bukidnon | Green | Strong | Semi-upright | Semi-pubescent | Pointed | Light green | Light green | Highly pubescent | Intermediate | Present | Green | Strong | Absent | Yellow | Purple | Purple | Present | Light brown | Circular | Absent |
| Gb64788bk | Bukidnon | Dark green | Strong | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Gb64784bk | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Light brown | Circular | Present |
| Gb61523bk | Bukidnon | Green | Weak | Semi-upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Medium | Absent | Yellow | Purple | Dark purple | Present | Mixture | Elliptic | Present |
| Gb64776bk | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Gb64777bk | Bukidnon | Dark green | Strong | Semi-upright | Semi-pubescent | Pointed | Light green | Light green | Highly pubescent | Erect | Present | Green | Weak | Absent | Yellow | Purple | Dark purple | Present | Pale yellow | Circular | Present |
| Gb64779bk | Bukidnon | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Light brown | Circular | Absent |
| Gb64780bk | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Light brown | Circular | Present |
| Gb64782bk | Bukidnon | Dark green | Strong | Upright | Smooth | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Elliptic | Present |
| Gb64785bk | Bukidnon | Green | Weak | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Present | Yellow | Purple | Light green | Present | Light brown | Elliptic | Present |
| Gb64781bk | Bukidnon | Green | Weak | Semi-upright | Semi-pubescent | Pointed | Dark green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Gb64775bk | Bukidnon | Green | Weak | Semi-upright | Semi-pubescent | Pointed | Green | Light green | Smooth | Intermediate | Present | Green | Medium | Absent | Yellow | Purple | Light green | Present | Mixture | Elliptic | Absent |
| Gb61536bk | Bukidnon | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Gb63368as | Agusan del sur | Dark green | Strong | Upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Kl2017-1ls | Lanao del sur | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Strong | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Wl2016-4ls | Lanao del sur | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Medium | Absent | Yellow | Purple | Light purple | Present | Dark brown | Circular | Present |
| Wl2017-4ls | Lanao del sur | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Gb62305mor | Misamis occidental | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Absent to very weak | Absent | Yellow | Purple | Light green | Present | Pale yellow | Circular | Present |
| Gb62019sar | Saranggani | Green | Weak | Upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Medium | Absent | Yellow | Purple | Light purple | Present | Pale yellow | Circular | Present |
| Gb60305sc | South Cotabato | Dark green | Strong | Semi-upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Weak | Absent | Yellow | Purple | Light green | Present | Light brown | Elliptic | Present |
| Gb2016-22zs | Zamboanga del sur | Dark green | Strong | Semi-upright | Highly pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Strong | Present | Yellow | Purple | Light green | Present | Light brown | Circular | Present |
| Brs2016-23zs | Zamboanga del sur | Dark green | Weak | Semi-upright | Smooth | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Medium | Present | Yellow | Purple | Purple | Present | Light brown | Circular | Present |
| Gb63370zs | Zamboanga del sur | Green | Weak | Semi-upright | Semi-pubescent | Pointed | Light green | Light green | Smooth | Intermediate | Present | Green | Medium | Absent | Yellow | Purple | Light green | Present | Dark brown | Elliptic | Present |

Figure 4 shows dark purple, purple, and light green types. At maturity, grain colors expressed were light brown, dark brown, yellow, and mixture. Brown to yellow hues of mature grains is commonly observed for cultivar types of *adlay*. The shape of mature grains was mostly circular, with populations GB61523BK, GB64782BK, GB64785BK, GB64775BK, GB60305SC, and GB63370ZS having elliptic grains. In contrast, Shen et al. (2019) reported a majority of oval-shaped grains among Chinese *adlay* populations, with the circular type comprising only 5.7% of the population set. Arora (1977) noted that varieties of the cultivated ma-yuen type have a variable grain shape from pear-shaped to spheroidal. Most populations have furrows on grains, with GB64779BK, GB61477BK, GB64775BK, and BK2016-003 having furrowless grains. Figure 5 shows examples of the presence and absence of furrows on immature grains. Mature grain features share similarities with Chinese *adlay* germplasm, having white, yellowish-white, gray-brown, dark brown,

and black husk color with a longitudinal groove on the ventral surface (Xi et al. 2016; Weng et al. 2022). Dark brown, light brown, and pale yellow husks with surface grooves or furrows were observed in the present set. However, no black seed coat was observed. Furrows, described as deep longitudinal grooves, were present in most of the populations in this study. In contrast, Shen et al. (2019) found that 85.1% of the mix populations of Chinese *adlay* have smooth bract surfaces.

Proportion of morphological types observed and estimates of morphological diversity

The Mindanao *adlay* populations showed some diversity in 13 of 20 morphological traits. Table 2 summarizes the proportion of observed morphotypes of variable traits and the respective calculated H' diversity index values.

Table 2. The proportion of observed morpho-types and calculated diversity index (H') values.

| Morphological trait | Observed type | Proportion | H' value |
|-------------------------------------|---------------------|------------|----------|
| Seedling color | Green | 76.5 | 0.3045 |
| | Dark green | 23.5 | |
| Intensity of anthocyanin coloration | Strong | 26.5 | 0.5261 |
| | Weak | 73.5 | |
| Plant growth habit | Upright | 67.6 | 0.5730 |
| | Semi-upright | 34.4 | |
| Plant growth habit | Upright | 67.6 | 0.5730 |
| | Semi-upright | 34.4 | |
| Leaf blade pubescence | Smooth | 5.9 | 0.7961 |
| | Semi-pubescent | 50.0 | |
| | Highly pubescent | 44.1 | |
| Leaf sheath color | Light green | 94.1 | 0.1249 |
| | Dark green | 5.9 | |
| Leaf-sheath pubescence | Smooth | 91.2 | 0.2716 |
| | Highly pubescent | 8.8 | |
| Leaf orientation | Erect | 5.9 | 0.1614 |
| | Intermediate | 94.1 | |
| Anthocyanin coloration of culm | Absent to very weak | 41.2 | 0.9172 |
| | Weak | 26.5 | |
| | Medium | 23.5 | |
| | Strong | 8.8 | |
| Culm glaucosity | Absent | 91.2 | 0.4306 |
| | Present | 8.8 | |
| Color of immature grains | Light green | 82.3 | 0.4180 |
| | Light purple | 5.9 | |
| | Purple | 8.8 | |
| Color of grains at maturity | Pale yellow | 58.8 | 0.7538 |
| | Light brown | 26.5 | |
| | Dark brown | 8.8 | |
| | Mixture | 5.9 | |
| Grain shape | Circular | 82.4 | 0.4242 |
| | Elliptic | 17.6 | |
| Presence of furrows on grain | Absent | 8.8 | 0.4306 |
| | Present | 91.2 | |



Figure 1. An upright A. (GB64779BK) and semi-upright B. (GB61477BK) plant growth habit



Figure 2. Anthocyanin coloration of the culm: A. Strong, B. Medium, C. Weak (GB64782BK), and D. Absent (GB64785BK)



Figure 3. Culm glaucosity: A. Present, B. Absent



Figure 4. Color of immature grain: A. Dark purple, B. Purple, and C. Light green



Figure 5. Grain furrows type: A. Presence and B. Absence

As noted in the above results, seven morphological traits were considered invariant among the populations. All populations share the same morphotype. For the variable traits, calculated Diversity Indices ranged from $H' = 0.1249$ (leaf sheath color) to $H' = 0.9172$ (anthocyanin coloration of culm). Six traits can be classified as medium or intermediate diversity. These are the intensity of anthocyanin coloration of culm, plant growth habit, culm glaucosity, color of immature grains, grain shape, and presence of furrows on grain. Glaucosity and anthocyanin in plants were considered valuable in plant growth responses. In durum wheat, Almarri et al. (2023) reported the significant contribution of plant glaucosity traits and coleoptile anthocyanin colorations. In this study, the traits were found to indicate considerable variability estimates. The populations, thus, can be considered for further evaluation of the observed variation.

Leaf blade pubescence, anthocyanin coloration of culm, and color of grains at maturity were classified as high diversity with H' values of 0.7961, 9172, and 0.7538, respectively. Singh et al. (2021) also reported multiple variations in leaf blade pubescence among indigenous Indian rice germplasm collections. Mekdad and Rady (2016) cited the potential functional role of plant leaf pubescence and waxes in protecting leaves from high temperatures and transpiration. The *adlay* populations in this study exhibited a variable type of leaf pubescence, which may be considered for evaluation under low-moisture and high-temperature environments. H' values for seedling color, leaf sheath color, leaf sheath pubescence, and leaf orientation can be classified as low, having values below the arbitrary values of 0.40. Mezghani et al. (2017) used the following range of values to classify the Shannon computed Shannon Index as High ($H' \geq 0.60$), Intermediate/Medium ($H' \leq 0.40 < 0.60$), and Low ($H' < 0.40$). The relatively low Diversity Indices obtained for the descriptors can be associated with the low number of observed types concerning the number of listed descriptors, which drags the value down upon standardization. Since the estimate is a function of the frequency of occurrence and several observed classes, highly skewed class distribution and fewer classes yield low estimates. Standardization of value further drags down the index as a correction to account for the known class(es) but not observed in the population.

Values, therefore, can indicate which classes dominate the population, occur in low frequency, and are not represented. Thus, the estimate measures the richness and evenness of the diversity structure (Zeleny 2021). Hence, the low-index characters had the most unobserved types relative to the known variability in the coix trait. In addition, one type significantly dominated over other observed types. The unrepresented and low-frequency classes could be identified as a priority in plant genetic resource diversity conservation. Forward exploration and collecting for the local *adlay* germplasm may take special attention for such types.

Though the current materials were composed of a small population from a defined geographic region in Mindanao island, with the majority coming from one province, Bukidnon, some degree of variability was expressed through qualitative morphological traits. Thirteen of 20 traits have at least two forms observed. However, Diversity Indices were generally limited, with low to intermediate values. The values were about the known descriptor list for *Coix* (UPOV 2012). Thus, not all morphotypes were observed in the current local materials, resulting in lower H' values after standardization. Only leaf pubescence and anthocyanin coloration of culm have all descriptor states observed in the set. Information on the morphological assessment of variability in *adlay* is quite limited. Shen et al. (2019) noted that the qualitative grain traits, total bract surface characteristics, total bract texture, and total bract shape area are among the significant sources of variation in a study of Chinese coix populations. For the local *adlay* populations, Wester (1922) noted that grain shapes ranged from spherical to oblong in populations from Zamboanga del Sur. The same grain shape types were observed in the present study. The color of mature grains observed was similar to the range of colors earlier reported by Wester (1922), but all earlier colors listed were currently not observed.

However, It should be noted that the current study had only two populations referenced from Zamboanga del Sur. Hence, the number of samples could be too few to capture other morphotypes possibly existing in the geographic subsection. He also noted seven distinct kinds of *adlay* in the region. Though it was not clear if all seven were of the starch types, the Subanen farmers named at least four populations. These are *Ginampay*, *Gulian*, *Pulot/Tapol* and *Linay* (BDP 2022). Except for *Linay*, the three farmers' varieties are included in this study. At the molecular level, Fu et al. (2019) similarly reported a low genetic diversity estimate based on AFLP among 139 *adlay* populations from China. In addition, morphological variations within populations were not strongly observed. Of the polymorphic traits observed, only the color of grains at maturity showed mixed types for two populations, GB61523BK and GB64775BK. The observed mixture of color types for the two populations could suggest the presence of variability within populations for the accessions characterized. Intra-population diversity coix was earlier noted by Shen et al. (2019) as prevalent among the Chinese germplasm based on SSR. A similar observation on within-population variability within coix

populations was earlier reported by Fu et al. (2019) using AFLP. Intra-population variability was similarly reported on other species like *Dactylis* L. based on AFLP (Sun et al. 2017), *Brassica rapa* L. using SSR (Phukan et al. 2020), and *Daucus* L. based on microsatellite marker (Maksylewicz and Baranski 2013). Within a population, variation is commonly expected in species with the cross or open nature of pollination (Sunil et al. 2016), such as coix. Using morphological markers, natural outcrossing in cultivated coix was estimated to range from 35.9% to 37.4% (Mello et al. 1995). The current result indicated potential inter-population genetic variability of the local starch *adlay*. However, procedural bias to eliminate possible observation errors could have obscured the detection of the presence of within-population variations in other morphological traits. In addition, genetic variability within populations may not have been clearly expressed in discrete qualitative morphological traits.

By and large, a certain degree of variability is present among the populations of cultivated *adlay* from Mindanao island. The importance of genetic diversity is a well-placed principle in crop agriculture. In crop variety improvement, it is an essential resource for developing commercial cultivars with the desired or preferred characteristics (Govindaraj et al. 2015). Diversity could yield valuable traits much sought in the quest for food sufficiency and sustainability (Onda and Mochida 2016). The observed genetic diversity in the current population set can therefore represent a subset of the genetic variability in the Philippine *adlay*, which can be further assessed for the potential use of the current morphological characteristics. Further exploration and collecting for the locally cultivated *adlay* may yield more variability as some earlier known types were not currently represented. In addition, assessment of genetic diversity at the molecular level may unravel genetic differences not visually observed by the phenotypic morphological characterization, including the possible presence of intra-population diversity.

Average weighted similarities of populations across all traits

Figure 6 shows the dendrogram of the multivariate clustering procedure for the 34 *adlay* populations following the agglomerative hierarchical method based on averaged weighted similarities. In general, the range of variability is narrow, with overall clustering achieved at a 0.50 similarity coefficient. Four sets of grouped populations were declared similar at 1.0 coefficient. That is, populations within the set have the same values across all traits. At 0.65 similarity coefficient, the populations can be grouped into 3 clusters with GB2016-22ZS, GB64777BK, and GB61477BK as outliers. These populations exhibited the most different morphotypes across the descriptors. Cluster I was composed of 21 populations with four groups having maximum similarity. Cluster II contained six populations,

while Cluster III included four populations. Cluster I may be subdivided into two groups, Cluster Ia and Cluster Ib. The similarity coefficients obtained in this study were similar to the earlier report of Dwipa et al. (2022) on coix populations from West Sumatra based on morphological characterization. Grouping by provenance sources is not evident. Populations from Bukidnon comprised 23 samples dispersed across the dendrogram, including the outliers. While populations from Lanao del Sur were grouped in Cluster IB, the Zamboanga del Sur collections were separately distributed to Cluster II, Cluster III, and as an outlier.

Ma et al. (2010), using microsatellite markers earlier, found an absence of association of clusters with provenance among the Korean *adlay* accessions. The same observation was reported by Ma et al. (2010) using microsatellite markers on Korean *adlay* accessions. They, however, found that Korean and Chinese populations grouped separately. Shen et al. (2019) found mixed results and concluded that geographic differences among Chinese *adlay* germplasm did not strongly influence genetic differences. On the other hand, Li et al. (2019) found that *adlay* populations collected in adjacent regions tend to cluster together regions brought about by frequent germplasm exchanges. The current results may indicate this possibility given that the provenances of samples were located in one geographic region, with some of the source provinces generally adjacent.

On the other hand, notable traits that distinguished the groupings were seedling color, Cluster I and Cluster III having mostly green while Cluster II mainly was dark green. Cluster I and III have mostly weak-intensity anthocyanin coloration, while Cluster II was mostly of strong intensity. Plant growth habit was mostly upright for Cluster I and II, while Cluster III was mostly semi-upright. Cluster I had a variable mixture of types of anthocyanin coloration of the culm, while Cluster II was mostly weak. Cluster III were all rated to be of medium anthocyanin culm coloration. Cluster I has the most color types of immature grains but predominantly light green.

Similarly, Cluster II was all light green immature grains, but light green and dark purple immature grain colors represented Cluster III. Cluster I and II had primarily light brown or pale yellow grain colors at maturity, while Cluster III was either dark brown or mixed colors. Cluster I and II had the mostly circular shape of mature grain, while Cluster III was mostly elliptic. Differentiation of *adlay* populations based on grain attributes has been demonstrated earlier. Xi et al. (2016) similarly noted the clustering of Chinese *adlay* populations according to grain characteristics such as grain color, surface characteristics, and kernel color. Similarly, Cong et al. (2023) grouped Vietnamese coix populations based on grain features, such as the presence of grain stripes and immature and mature grain colors.

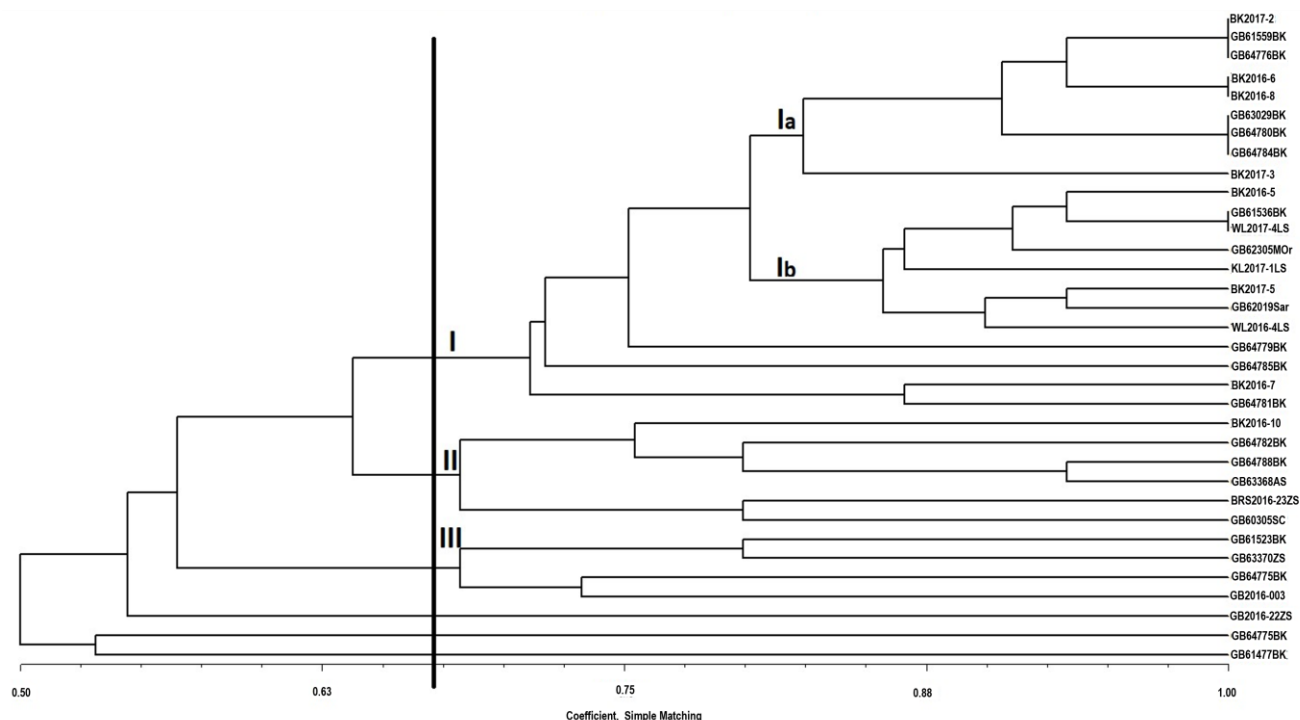


Figure 6. Dendrogram of 34 traditional *adlay* populations from Mindanao Provinces, Philippines

Starch-forming traditional *adlay* populations from Mindanao provinces in the Philippines were described for qualitative morphological traits. Based on observed morphotypes, phenotypic diversity of the local *adlay* was found to be generally intermediate, with some traits being high following the Shannon Diversity Index. Within a population, variability was not prevalent but noted in one of the polymorphic traits. In addition, the local *adlay* populations can be clustered into three main groups with a few small groups within based on the degree of similarities in morphotypes across the set of qualitative descriptors considered. The association of morphotypes to provenances was not evident. Further germplasm collecting, marker-based characterization, and evaluation are suggested to unravel the potential agronomic value of some traits and further understand the genetic structure of the local cultivated *adlay* populations.

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