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Genetic Relationships of Cultivated Avocado Germplasm in Gorontalo Based on Qualitative Traits

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Abstract: The avocado (*Persea americana*), a recognized superfood and valuable horticultural commodity, exhibits significant morphological diversity due to long-term adaptation to various habitats, enhancing biodiversity. This study analyzed the diversity and genetic relationships of cultivated avocados in Gorontalo Regency. Using the Descriptors for Avocado (*Persea* spp.), 25 qualitative morphological traits were characterized, and a phenetic analysis of 17 accessions was conducted using UPGMA with Jaccard's Coefficient. Accessions were collected from six villages across four sub-districts: Dulamayo Barat, Dumati, Hepuhulawa, Kayubulan, Kayumerah, and Tridarma. Results revealed substantial diversity in traits such as tree shape, and the morphology of stems, leaves, fruits, and seeds. Phenetic analysis grouped the accessions into two clades: Clade A, comprising 15 accessions in two clusters (similarity index: 64.7-29.0%), and Clade B, containing two accessions in one cluster (similarity index: 64.7%). Clades A and B exhibited a distant relationship, with a similarity index of 23.6%. Conserving the diverse avocado germplasm is essential for safeguarding genetic resources and supporting breeding programs to develop superior varieties in Gorontalo.

Keywords: Characterization; Exploration; Morphology; *Persea americana*; Gorontalo

Introduction

The avocado, scientifically known as *Persea americana*, is one of the oldest flowering plants in the Lauraceae family and the order Laurales. Native to Mexico and Central America, it has spread to various regions of the world, from tropical to subtropical areas (Kuswandi et al., 2017; Phaw & Aye, 2017; Ranjitha et al., 2021). The avocado is widely cultivated for its creamy-textured flesh with a unique, nutrient-rich taste (Augustine et al., 2021; Awachare et al., 2023), making it one of the superior horticultural commodities (Verti et al., 2021).

Avocado flesh contains up to 30% fat and 4% protein, as well as high levels of vitamins (especially vitamins E and C) and minerals (Cu and Fe), but is relatively low in carbohydrates (Ge et al., 2017; Hurtado-Fernández et al., 2018). Interestingly, avocado fat contains no cholesterol and has been observed to reduce LDL levels by 22% while simultaneously increasing HDL levels by 11%. The avocado plant is also rich in bioactive compounds that act as antioxidants, anticancer agents, antimicrobials, anti-inflammatory agents, and antidiabetics (Bhuyan et al., 2019). Due to these properties, avocado has been widely used in the pharmaceutical, cosmetic, and culinary industries (Ge et al., 2017).

Research on avocado exploration and morphological characterization has been previously conducted by Ge et al. (2017) in Hainan Province, China; Phaw & Aye (2017) in Myanmar; Abraham et al. (2018) in Ghana; Juma et al. (2020) in Tanzania; and Ranjitha et al. (2021), Augustine et al. (2021), and Awachare et al. (2023) in various locations in India. Similar research on the morphological characterization of avocado in various provinces of Indonesia has also been reported by Marsigit (2016) in Central Java and Yogyakarta, Kuswandi et al. (2017) in Solok Regency, West Sumatra, Verti et al. (2021) on Bangka Island, Bangka Belitung Islands, and Ishaq et al. (2023) in West Java. The morphological characteristics of all the accessions found in these studies demonstrate the high genetic diversity

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of avocado varieties across different regions, including Indonesia.

Plant adaptation to different environmental conditions and growing locations over a long period can result in unique morphological traits in the species (Baderan et al., 2024). These unique morphological traits often result in variation at the subspecies level. Assessing diversity through morphological markers plays a crucial role in describing and characterizing germplasm (Ranjitha et al., 2021). These easily observable phenotypic traits serve as an initial indicator for superior variety plant breeding programs.

To date, information regarding the existence of germplasm and the morphological diversity of cultivated avocados in Sulawesi, especially in the Gorontalo region is unavailable. However, the availability of such information would significantly support and optimize future conservation efforts for genetic resources in the Gorontalo region, as well as provide a foundation for plant breeding programs to develop superior varieties or create new varieties. The provision of information on the existence and diversity of plant germplasm can be achieved through research that explores, identifies, and characterizes traits, as well as analyzes the relationships between plant accessions. Given the unique geological, ecological, and geographical characteristics of Sulawesi Island, particularly Gorontalo Regency, the adaptation of avocado plants in this region may result in morphological trait variations that differ from those found in other parts of Indonesia or other countries. This study aims to analyze the diversity and genetic relationships of cultivated avocados in the Gorontalo Regency.

Method

This research was conducted from June to September 2024. The study area focused on Gorontalo Regency, as the largest avocado-producing center in the Gorontalo Province. This research employed an exploration method to locate observation objects in a particular area, along with a qualitative descriptive method to describe the morphological diversity of cultivated avocado germplasm in Gorontalo Regency and its genetic relationships. The research procedure is described as follows (Figure 1).

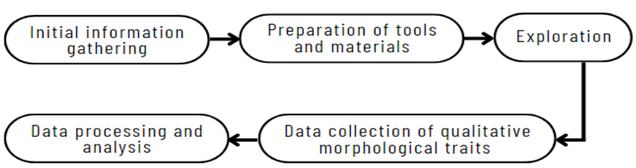

Figure 1. Research flowchart

Exploration

The exploration of cultivated avocado germplasm began by gathering information from fruit traders, local communities, and other informants regarding the location of avocado orchards or where avocado plants were found in Gorontalo Regency. The research team then visited the cultivated avocado planting sites. Sampling locations were recorded to facilitate tracking. Newly planted avocado plants aged 2-3 years were excluded from the study.

Data Collection

Data collection was carried out purposively, selecting one mature or fruit-bearing avocado tree (if available) at each sampling location that had reached full maturity. The characterization of cultivated avocado germplasm was conducted by identifying 25 qualitative morphological traits in the stem, leaf, fruit, and seed organs, based on the Descriptors for Avocado (*Persea* spp.) (IPGRI, 1995). The fine, short hairs on both the upper and underside of the leaf were examined using a microscope, while the remaining 23 characteristics were observed by the naked eye or through touch. All cultivated avocado accessions were replicated thrice for qualitative morphological characterization. All observed qualitative morphological traits were recorded and documented. Additionally, the coordinates of the avocado's growing location were recorded using GPS.

Data Processing and Analysis

All data obtained from the qualitative morphological characterization were assigned a 1-0 scoring system based on the presence or absence of a trait to facilitate further phylogenetic analysis (Baderan et al., 2024). The 1-0 matrix was then analyzed using Multivariate Statistical Package (MVSP) software version 3.2 to construct a dendrogram showing the phylogenetic relationships among avocado accessions based on similarity indices, using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Jaccard's Coefficient.

Result and Discussion

Location of Accessions

The exploration of cultivated avocados in Gorontalo Regency identified 17 accessions distributed across 6 villages in 4 districts: Dulamayo Barat Village in Telaga District, Dumati Village in Telaga Biru District, Hepuhulawa, Kayubulan, and Kayumerah Villages in Limboto District, as well as Tridarma Village in Pulubala District (Table 1). Most of the avocado accessions are cultivated by local communities in home gardens. The highest number of cultivated avocado accessions was found in Limboto District (9 accessions), while the lowest was in Telaga District (1 accession).

Tabel 1. Location and accession code of cultivated avocado in Gorontalo Regency

Accession Code	Location	Coordinates
DMY01	Dulamayo Barat Village, Telaga District	0°36'22"N 123°01'45"E
DUM01	Dumati Village, Telaga Biru District	0°41'52"N 123°03'12"E
DUM02		0°41'51"N 123°03'12"E
DUM03		0°41'51"N 123°03'12"E
DUM04		0°41'50"N 123°03'12"E
HEP01	Hepuhulawa Sub-district, Limboto District	0°37'15"N 122°59'21"E
HEP02		0°37'15"N 122°59'20"E
KBL03	Kayubulan Sub-district, Limboto District	0°37'01"N 122°58'43"E
KBL04		0°37'01"N 122°58'43"E
KBL05		0°37'01"N 122°58'43"E
KBL06		0°37'02"N 122°58'43"E
KBL07		0°37'01"N 122°58'43"E
KBL08		0°37'01"N 122°58'43"E
KMR01	Kayumerah Sub-district, Limboto District	0°37'52"N 122°58'32"E
TDM01	Tridarma Village, Pulubala District	0°38'36"N 122°48'48"E
TDM02		0°38'37"N 122°48'49"E
TDM03		0°38'36"N 122°48'49"E

A total of 25 qualitative morphological traits of stem, leaf, fruit, and seed organs were identified based on the Descriptors for Avocado (*Persea* spp.). Avocado showed very high morphological diversity, with 4 out of 25 observed qualitative traits being dimorphic (surface of young twig, leaf margin, petiole groove, and fruit apex position), while the remaining 21 traits were polymorphic.

Tree and Stem Morphology

The tree (canopy) and stem forms of 17 cultivated avocado accessions exhibited diverse morphological traits (Figure 2). Five tree shapes were identified, with the circular form being the most dominant (35.29%), followed by pyramidal, columnar, and rectangular forms (each 17.65%), and obovate (11.76%). Most accessions had very rough trunk surfaces (52.94%), while 41.18% had rough trunk surfaces and the remaining 5.88% had smooth trunk surfaces. The branching pattern of these 17 avocado accessions was dominated by ascendant and irregular forms (each 35.29%), while the remainder were axial (23.53%) and verticillate (5.88). Young twigs were generally green in color (58.82%), while others showed shades of green with slight orange or reddish hues (29.41%), yellow-orange (5.88%), and red (5.88%). The surface of young twigs was mostly glabrous (smooth and hairless) (58.82%), while the remainder were pubescent with short and soft hairs (41.18%).

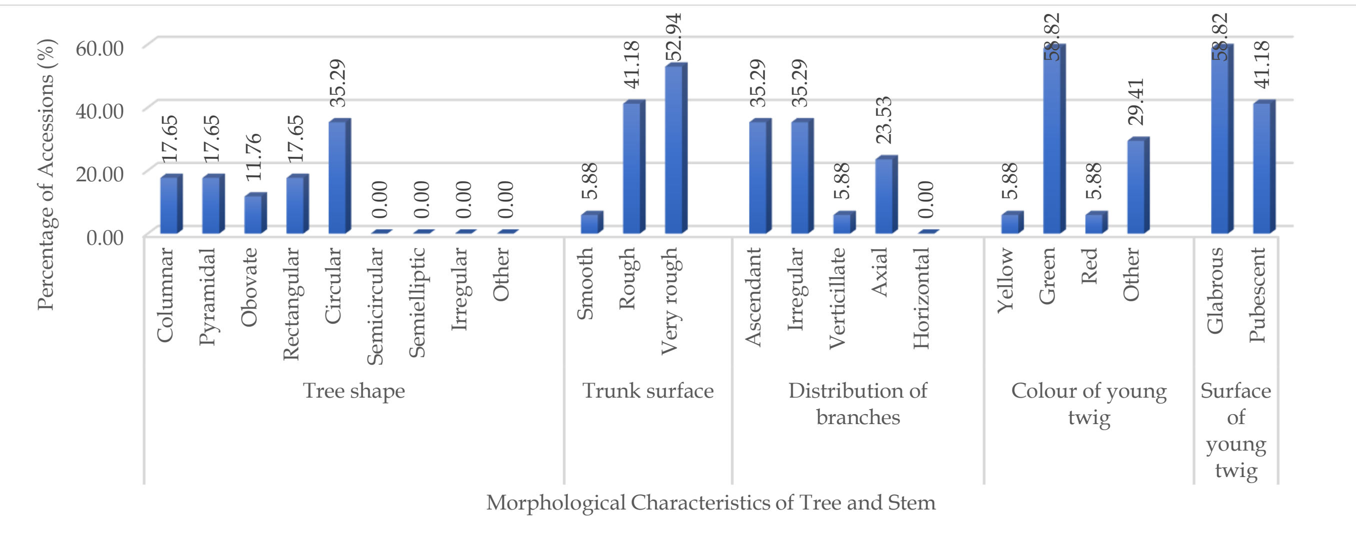

Figure 2. Percentage of tree and stem shape characteristics of 17 cultivated avocado accessions in Gorontalo Regency

These findings were similar to Juma et al. (2020), who reported that Tanzanian avocados mostly had very rough trunk surfaces (38.9%). In contrast, Abraham et al. (2018) and Awachare et al. (2023) reported that most avocado accessions in Ghana and India (64.2% and 45.83%, respectively) had rough trunk surfaces. Based on the leaf color of young twigs, most of the cultivated avocado accessions in Gorontalo Regency belong to the Mexican race (*P. americana* var. *drymifolia*) characterized by young green leaves, followed by the Guatemalan race (*P. americana* var. *guatemalensis* L. WMS.) characterized by green leaves with a reddish tinge (Hurtado-Fernández et al., 2018). The cultivated avocado varieties in Gorontalo Regency contradict Kuswandi et al. (2017), who stated that the Mexican and Guatemalan races are suitable for cultivation in subtropical regions, while the West Indian race is more suitable for tropical areas.

Leaf Morphology

The leaves of the cultivated avocado accessions in Gorontalo Regency also displayed a high diversity of morphological traits (Figure 3). Among the 17 accessions, 10 accessions (58.82%) exhibit oval-shaped leaves, followed by ovate and oblong-lanceolate shapes (11.76% each), and narrowly obovate, lanceolate, and oblong shapes (5.88% each). The leaf bases of the observed accessions were predominantly acute (88.24%) and the rest were obtuse (11.76%). The apex of the leaves was mostly acute (47.06%), followed by intermediate (29.41%), obtuse (17.65%), and very acute (5.88%) forms. The leaf margins were primarily entire (76.47%), meaning smooth without waves. Mature leaves were generally dark green (47.06%) and green (41.18%), with only 11.76% having light green color. The degree of greenness is influenced by the chlorophyll content in the leaves, which correlates with photosynthetic capacity. The darker the green color of the leaves, the higher the chlorophyll content, leading to increased photosynthetic activity (Urry et al., 2020). Most avocado leaves have a semi-hard or semi-rigid texture (70.59%), while the rest were hard (17.65%) and soft (11.76%). The petioles of the leaves typically had grooves (94.12%). Additionally, both the upper and lower surfaces of the leaves displayed hairs (pubescence), with the underside mostly falling into intermediate and dense intensity categories (41.18% each), while the upper surface was typically sparsely haired (47.06%).

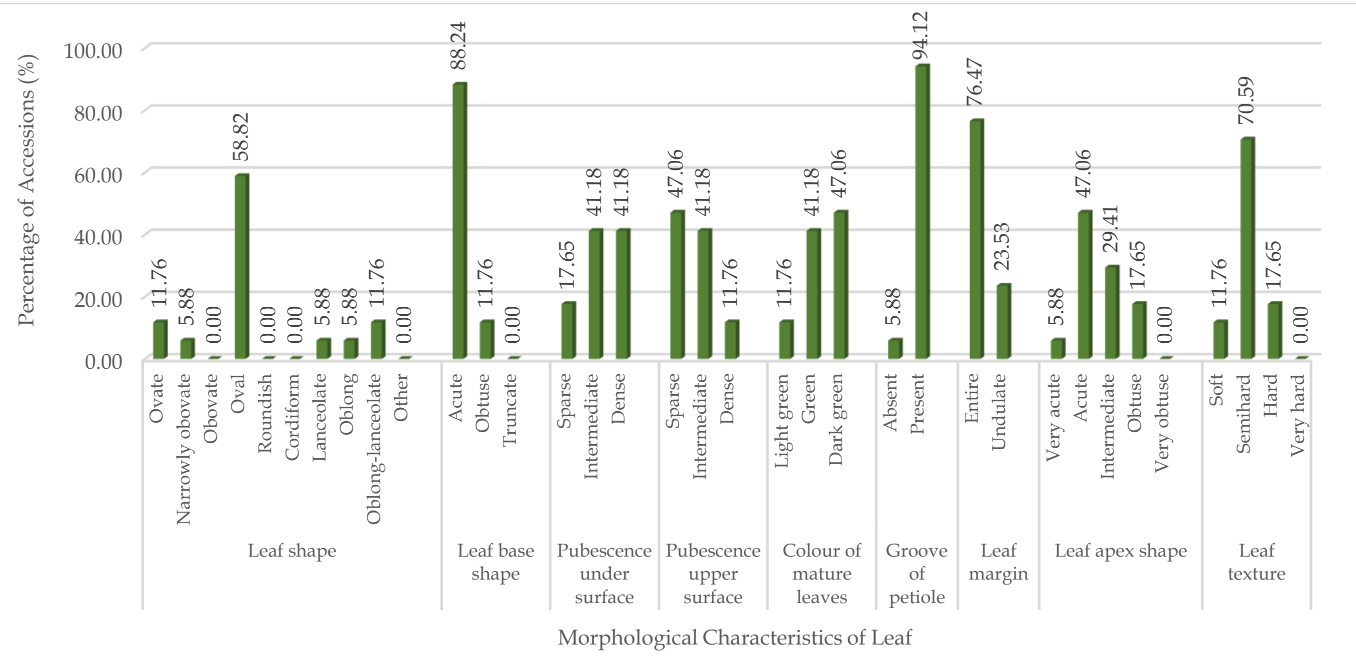

Figure 3. Percentage of leaf characteristics of 17 cultivated avocado accessions in Gorontalo Regency

Fruit and Seed Morphology

Out of the 17 cultivated avocado accessions, only 9 were found bearing fruit at the sampling locations, and all were in the fruit development phase. These 9 accessions exhibited diverse morphological traits in both fruit and seeds (Figure 4). Of the 10 avocado fruit shapes described by IPGRI (1995), only 5 shapes were found at the sampling locations. The avocado fruit was predominantly narrowly obovate in shape (55.56%), while the remaining were spheroid, high spheroid, pyriform, and rhomboidal (11.11% each). These results were different from Awachare et al. (2023) who reported that most Indian avocados had obovate (26.38%) followed by narrowly obovate (18.05%), clavate (16.67%), and spheroid (5.56%) fruit shape. The fruit base was mostly depressed (44.44%), while the apex was mostly inflated (66.67%). The apex was generally centrally located on the fruit (66.67%), as was the pedicel, which was mostly rounded (55.56%). The fruit typically lacked ridges (55.56%), had intermediate surface roughness (66.67%), and showed a weak to medium gloss (44.44% each). When ripe, the fruit was generally green (77.78%). The seed shape in the nine avocado accessions was predominantly broadly ovate, with a flattened base and rounded apex, or a flattened base and conical apex (33.33% each).

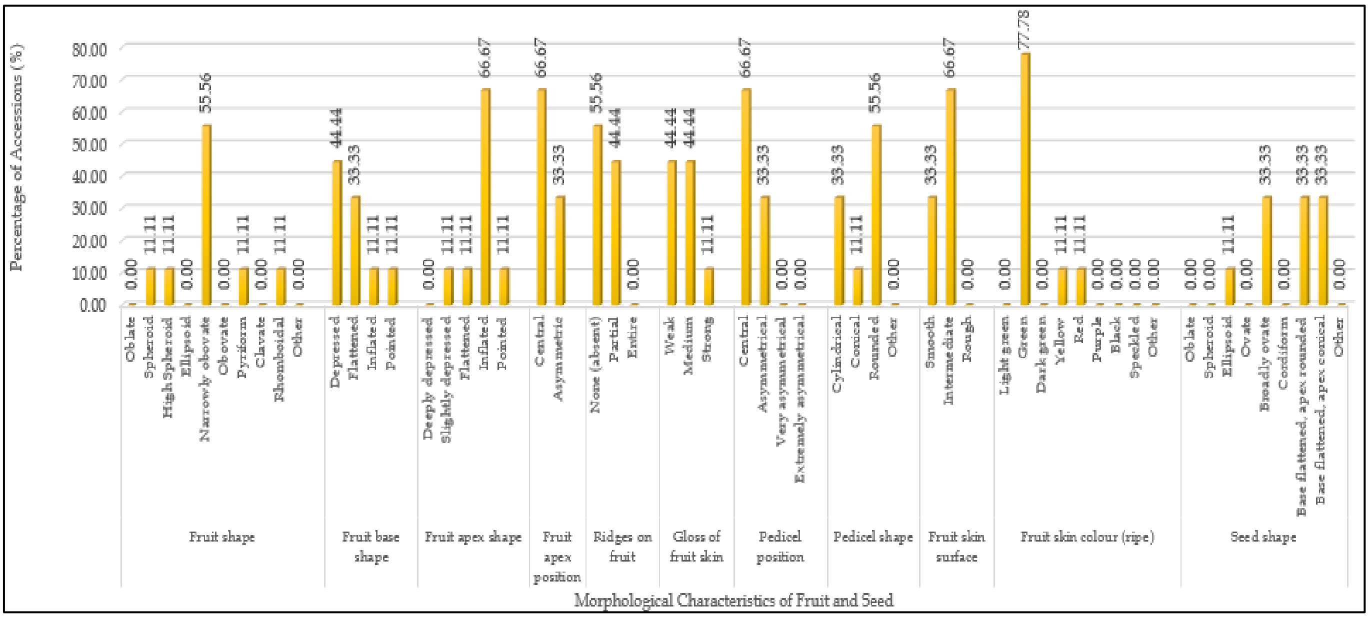

Figure 4. Percentage of fruit and seed characteristics of 9 cultivated avocado accessions in Gorontalo Regency

Phenetic Analysis

The morphological characters data in the form of a 1-0 matrix was used for phenetic analysis using UPGMA with Jaccard's Coefficient. The phenetic analysis in this study utilized data from 14 qualitative morphological characters of the tree canopy, trunk, and leaves, as complete data for the fruit and seeds of all accessions were unavailable. The UPGMA analysis with Jaccard's Coefficient for the 17 avocado accessions was used to construct a dendrogram based on similarity indices (Figure 5). The similarity matrix of the 17 cultivated avocado accessions is presented in Table 2.

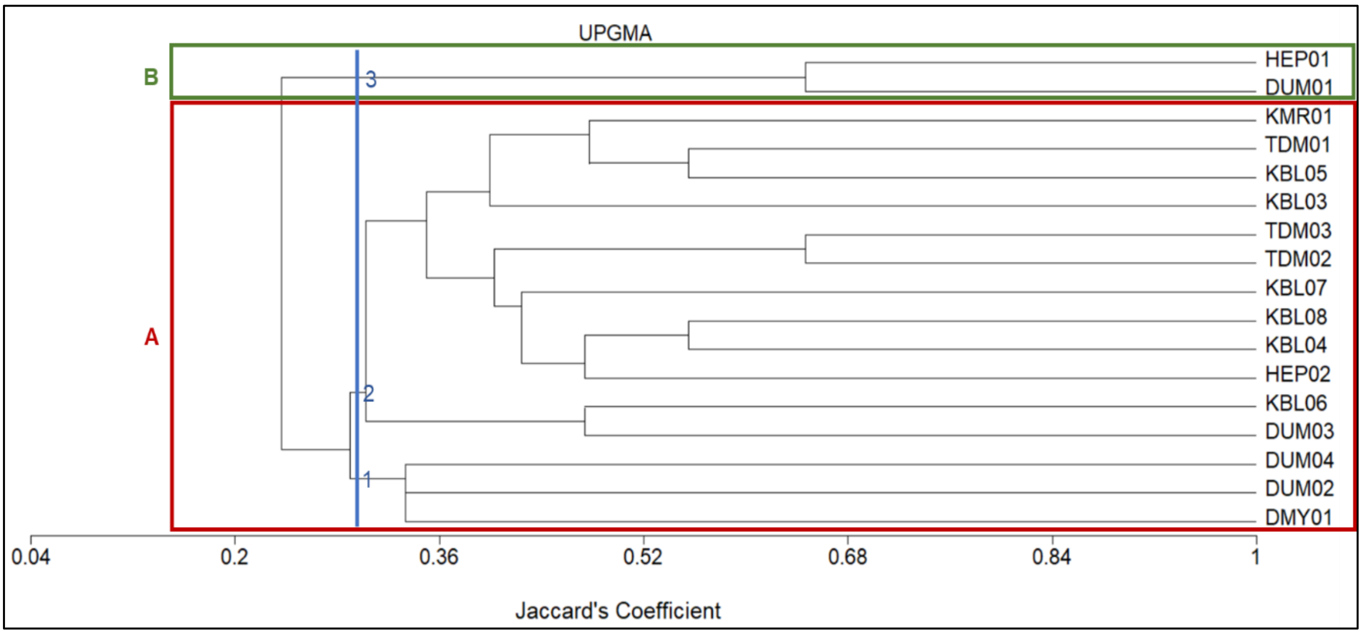

Figure 5. Dendrogram of clustering 17 avocado accessions based on morphological character similarity

Table 2. The similarity matrix of the 17 cultivated avocado accessions in Gorontalo Regency

	DMY01	DUM01	DUM02	DUM03	DUM04	HEP01	HEP02	KBL03	KBL04	KBL05	KBL06	KBL07	KBL08	KMR01	TDM01	TDM02	TDM03
DMY01	1.000																
DUM01	0.120	1.000															
DUM02	0.333	0.120	1.000														
DUM03	0.333	0.217	0.217	1.000													
DUM04	0.333	0.217	0.333	0.273	1.000												
HEP01	0.167	0.647	0.217	0.120	0.333	1.000											
HEP02	0.333	0.273	0.217	0.273	0.273	0.333	1.000										
KBL03	0.217	0.167	0.273	0.217	0.273	0.217	0.217	1.000									
KBL04	0.400	0.167	0.400	0.333	0.333	0.273	0.474	0.400	1.000								
KBL05	0.333	0.400	0.333	0.333	0.474	0.400	0.400	0.400	0.333	1.000							
KBL06	0.273	0.273	0.273	0.474	0.217	0.167	0.273	0.217	0.217	0.474	1.000						
KBL07	0.400	0.273	0.217	0.474	0.333	0.120	0.400	0.273	0.474	0.333	0.333	1.000					
KBL08	0.400	0.217	0.273	0.217	0.400	0.400	0.474	0.333	0.556	0.400	0.217	0.400	1.000				
KMR01	0.167	0.217	0.333	0.217	0.217	0.333	0.474	0.400	0.474	0.400	0.333	0.167	0.400	1.000			
TDM01	0.217	0.333	0.333	0.273	0.217	0.333	0.474	0.400	0.333	0.556	0.400	0.217	0.333	0.556	1.000		
TDM02	0.273	0.167	0.273	0.273	0.217	0.167	0.333	0.333	0.400	0.400	0.474	0.333	0.474	0.474	0.474	1.000	
TDM03	0.273	0.167	0.273	0.217	0.167	0.167	0.333	0.217	0.474	0.273	0.273	0.400	0.474	0.333	0.333	0.647	1.000

The similarity index may imply a genetic relationship between avocado accessions. A high similarity value indicates a close genetic relationship between them (Putri et al., 2023). The result of the phenetic analysis (Figure 5) shows that the 17 cultivated avocado accessions in Gorontalo Regency can be grouped into two clades with three clusters. Clade A consists of 15 accessions, which are divided into 2 closely related clusters (with similarities ranging from 64.7 to 29.0%). Cluster 1 comprises DMY01, DUM02, and DUM04, with a similarity among them of 33.3% each. Cluster 1 is closely related to Cluster 2, which contains 12 accessions, with similarities ranging from 64.7 to 30.2%. Meanwhile, Clade B consists of one cluster, which includes only two accessions (DUM01 and HEP01), with a similarity of 64.7%. Clade A and Clade B are distantly related, with a similarity of only 23.6% (Table 2). Clade B (HEP01 and DUM01) differs from all members of Clade A, particularly in leaf characteristics, including leaf shape, leaf base shape, and mature leaf color. Accessions with high similarity within Clusters 1, 2, and 3 share many similar qualitative traits, making them suitable for crossbreeding to obtain desired qualitative characteristics in breeding programs. However, accessions with unique fruit shape traits that are rare in Gorontalo Regency, such as KBL06 and DUM03, should be promoted for cultivation among the community as part of conservation efforts.

Conclusion

Based on the results of the study, it can be concluded that the 17 cultivated avocado accessions in Gorontalo Regency exhibit significant diversity in their qualitative characteristics, including variations in tree shape, as well as trunk, leaves, fruits, and seeds morphology. The phenetic analysis grouped the 17 accessions into two clades with three clusters: Clade A consists of 2 clusters and includes 15 accessions with a similarity index ranging from 64.7 to 29.0%, and Clade B consists of one cluster which includes only two accessions with similarity index of 64.7%. Clade A and Clade B show a distant relationship with a similarity index of only 23.6%.

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Author Contributions

M.J. designed the research, supervised the entire process, and wrote the manuscript; F.F. collected and analyzed the data; I.H.H, N.F.P, and T.I. conducted the exploration and collected the data.

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Conflicts of Interest

The authors declare that they have no conflict of interest regarding the research or the research funding.

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