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Research Article

Diverse Morphology and Anatomy of *Citrus* Spp. (Orange) in South Sulawesi, Indonesia Plantations: A Comprehensive Study

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Abstract

Background and Objective: South Sulawesi, one of the Indonesian provinces, is a producer of oranges with various varieties grown extensively for export and domestic use. Information about the diversity of oranges is crucial for plant breeding and germplasm conservation. This study aims to analyze the diversity of oranges from several plantation centers in South Sulawesi based on morphological and anatomical characteristics. **Materials and Methods:** Orange leaf samples were collected from five plantation locations in South Sulawesi, namely Pangkep, Sidrap, Bantaeng, North Luwu and Selayar Regencies. The morphological characteristics were identified using descriptors from the International Plant Genetic Resources Institute and Tjitrosoepomo. The anatomical characteristics were identified by preparing stomata slides observed under a microscope at a magnification of 200-400x. Similarity analysis between orange varieties was conducted using the NTSYS software and presented in the form of a dendrogram. **Results:** The results of the diversity analysis of 13 orange varieties showed morphological variability in tree form and leaf shape, while anatomical characteristics showed variability in stomata size and stomata index. The similarity analysis showed that morphological characteristics formed clusters consisting of seeded selayar (SB), kaffir lime (NN), JC-selayar (JS), selayar-selayar (SS), batu (B), japansche citroen (JC) and dekopon (D) varieties, which had a 75% similarity with siam (SI) and sweet santang (SM) varieties. Meanwhile, the anatomical cluster analysis showed that the JC and SM orange varieties had a 79% similarity with the D variety. **Conclusion:** The dendrogram diagram can serve as a basis for determining desired plant traits in plant breeding activities.

Key words: Breeding, local germplasm, similarity index, Sulawesi *Citrus* species

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oranges are fruit plants that belong to the Rutaceae family and are major fruits traded worldwide¹⁻³. According to statistical data, nearly 60% of global production of major fruits comes from ten countries, namely China, India, Brazil, the United States, Indonesia, the Philippines, Mexico, Turkey, Spain and Italy⁴. Indonesia has a high diversity of oranges due to the tropical climate that supports the growth and plantation of various types of oranges⁵. In Indonesia, oranges are a favorite fruit highly sought after by consumers and almost every province in the country has orange plantation areas. One of these provinces is South Sulawesi, a major orange producer with a wide range of varieties cultivated for both domestic use and export, orange plantation also contributes to the agricultural economy in the region⁶.

The orange species have an extraordinary ability in crossbreeding and producing intra or intergeneric hybrids. In orange plants, apomixis is a common process that allows hybrid propagation through nucellar embryos. This phenomenon poses a challenge for botanists and agronomists who have been striving for centuries to identify orange varieties and define orange taxa. One of the main reasons is that orange taxa often exhibit overlapping morphological characteristics and transitional forms between species frequently occur⁵. Such extensive variability results from field selection, propagation and the diffusion of selected varieties in different plantation areas throughout the year⁷. Information on diversity is necessary for determining genetic relationships, characterizing germplasm, breeding programs, taxonomy and registration of new varieties/cultivars^{8,9}. Characterization activities for the existing diversity of orange types are needed as an initial step to ensure the accuracy of the utilized varieties.

Morphological characterization involves studying visible traits¹⁰. Information obtained from the characterization is crucial as it provides a basis for accurate identification, classification of varieties and differentiation of local orange varieties from those grown worldwide. Understanding the anatomical structure of the orange is equally important. Anatomical analysis involves studying the arrangement, cellular structure and tissue organization of plants¹¹. Morphological and anatomical characterization are essential for understanding the diversity and uniqueness of local orange varieties; they can also assist in breeding programs¹². By identifying and understanding the diversity within and among orange varieties, breeders can select parents with specific morphological or anatomical traits to develop

improved varieties¹³. Accurate morphological and anatomical characterization is crucial for the conservation of orange germplasm^{14,15}.

South Sulawesi is home to a rich diversity of orange varieties, some of which may be unique to the region⁶. The characterization of oranges in South Sulawesi can contribute to the knowledge of orange diversity¹⁶. This information is valuable for researchers, extension workers and policymakers involved in orange-related studies and programs. The morphological and anatomical characterization of the orange can also facilitate marketing and branding efforts. By identifying distinctive morphological features, farmers can differentiate their products in the market and cater to specific consumer preferences. This creates opportunities for value-added products and marketing strategies that highlight the unique characteristics of South Sulawesi orange. There is a high diversity within the genus in terms of morphology and anatomy and each species has distinguishing characteristics that set it apart¹⁷. To identify the differences in these species' characteristics, a characterization approach is needed¹⁸. Research on the morphological and anatomical characteristics of various orange varieties grown in South Sulawesi is still limited. Based on preliminary observations, there are 13 orange varieties cultivated in five orange plantation centers in South Sulawesi. Therefore, this research aims to characterize these 13 orange varieties in terms of morphology and anatomy.

MATERIALS AND METHODS

Sample collection: This research was conducted from March to December, 2022. The orange leaf samples were collected from five orange trees, with ten leaves per tree for each variety gathered from five citrus plantations in South Sulawesi (Table 1). Pangkep Regency with pomelo *Citrus maxima* (Burm.) Merr., Sidrap Regency with lime *Citrus aurantifolia* L. and kaffir lime *Citrus hystrix* D.C, Bantaeng Regency with mandarin orange *Citrus reticulata* Blanco, North Luwu Regency with siam orange *Citrus nobilis* Lour, honey tangerine *Citrus reticulata* and dekopon *Citrus reticulata* Shiranui and Selayar Regency with Selayar tangerine *Citrus reticulata* L. and *Japansche citroen* (JC) *Citrus limonia* Osbeck. Morphological identification was conducted at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University. Leaf anatomy analysis was carried out at the Microbiology Laboratory of the Research and Development Agency for Environment and Forestry, Makassar.

Table 1: Orange varieties collected in five orange plantation centers in South Sulawesi, Indonesia

Location	Variety	Sample code	Geographical coordinates	Altitude (m a.s.l.)
Ma'rang, Pangkep	Red pomelo	M	Latitude S-4°42' "Longitude E 119°34"	32
	White pomelo	P		
	Sweet pomelo	G		
Pitu Riase, Sidrap	Lime	N	Latitude S-3.84° "Longitude E 119.81°	205
	Kaffir lime	NN		
	Batu orange	B		
Bissappu, Bantaeng	Sweet santang	SM	Latitude S-2°50' "Longitude E 120°19"	17
Malangke Barat, Luwu Utara	Siam orange	SI		
	Dekopon	D		
	Seeded selayar	SB	Latitude S-6°8'1" Longitude E 120°27"	268.5
	Selayar-selayar	SS		
	JC-selayar	JS		
Bontomatene and Bontona Saluk, Selayar	Japansche citroen	JC		

Identification of morphological characteristics: The identification of morphological characteristics of orange plants was conducted using descriptors from the International Plant Genetic Resources Institute¹⁹ and Suariaa *et al.*²⁰. Morphological characteristics measured included qualitative and quantitative traits. Qualitative characters included tree form, stem shape, stem growth direction, branching pattern on the stem, branch growth direction, leaf attachment (lamina), leaf shape (circumscription), leaf apex, leaf base, leaf venation, leaf margin, leaf parenchyma, leaf color, leaf surface, leaf arrangement on the stem (phyllotaxis), leaf wing, leaf petiole wing width and leaf petiole wing shape. Quantitative characteristics included average tree height, average stem diameter, leaf length, leaf width, leaf thickness and petiole length. Similarity analysis among orange varieties was performed by processing the morphological data using the Numerical Taxonomy and Multivariate Analysis System (NTSYS)Spc 2.10e software. The results of the similarity analysis were presented in the form of a dendrogram.

Identification of anatomical characteristics: The upper and lower leaf surfaces were treated with acetone while the leaves were still on the tree. Stomatal preparations were examined using a microscope (Nikon 119c Tokyo Japan) at magnifications ranging from 200 to 400x. The anatomical traits observed encompassed the stomata arrangement, stomatal types, stomatal length and width, stomatal size, stomatal index, upper and lower epidermis cell characteristics, upper and lower epidermis cell wall structure, stomatal guard cell morphology, trichome arrangement and form, stomatal opening and stomatal distribution pattern. Photographs of the observed samples were taken. Stomatal size (SS) was quantified using the following formula²¹:

$$SS = L \times B \times K$$

Where:

- L = Length
- B = Width
- K = Franco's constant (0.79)

Stomatal index (SI) is calculated based on the formula as follows²²:

$$SI (\%) = \frac{S}{S + E} \times 100$$

Where:

- S = Number of stomata
- E = Number of epidermal cells

Statistical analysis: The data obtained from the observation of morphological and anatomical characteristics were analyzed descriptively by presenting the morphological and anatomical features of all orange varieties in tables and figures. The similarity analysis between orange varieties was conducted by processing the data of morphological and anatomical characteristics using the Numerical Taxonomy and Multivariate Analysis System (NTSYS) Spc 2.10e software.

RESULTS AND DISCUSSION

Morphological characteristics: Morphological characterization involves the observation of morphological characteristics of plants based on both qualitative and quantitative properties. Observations were conducted on 24 characteristics. The qualitative characteristics observed included tree shape, stem shape, stem growth direction, branching pattern on the stem, branch growth direction, leaf attachment (lamina), leaf shape (circumscription), leaf apex, leaf base, leaf venation, leaf margin, leaf mesophyll, leaf color,

leaf surface, leaf phyllotaxis, leaf petiole wing, petiole wing width and petiole wing shape. Quantitative characteristics involved observing morphological traits based on size or the number of observed properties using appropriate units. The quantitative characteristics observed were average tree height, average stem diameter, leaf length, leaf width, leaf thickness and leaf petiole length.

Tree shape: Ellipsoid tree shape was found in the following orange varieties: Seeded selayar (SB), JC-selayar (JS), selayar-selayar (SS), red pomelo (M), white pomelo (P), sweet pomelo (G), batu orange (B), kaffir lime (NN) and dekopon (D). Meanwhile, the obloid tree shape was found in Japanshe citroen (JC), siam orange (SI), sweet santang (SM) and lime (N) citrus varieties (Fig. 1a-m).

Shape, growth direction and branching of the stem: In general, the 13 orange varieties have a round stem shape (teres) with an upright growth direction (erectus). The branching of the sympodial stem, which is the main stem, is difficult to determine as it may cease its growth or exhibit slower and smaller growth compared to its branches (Fig. 1a-m).

Branch growth direction: All varieties have an upright branch growth direction (fastigiatus). The angle between the stem and branches is very small, so the branch growth direction is slightly slanted upwards only at the base, but further up it is almost parallel to the main stem (Fig. 1a-m).

Leaf attachment (lamina): All orange varieties are classified as brevipetiolate, which means that the leaf stalk is shorter than the leaf blade (Fig. 2a-k).

Leaf shape: The leaf shapes of the 13 orange plant varieties vary. The ovate leaf shape was identified in seeded selayar (SB), JC-selayar (JS), selayar-selayar (SS), batu orange (B), siam orange (SI), sweet santang (SM), kaffir lime (NN) and dekopon (D). The reverse ovate leaf shape (obovatus) was found in red pomelo (M), white pomelo (P), sweet pomelo (G) and Japanshe citroen (JC). These leaf shapes are ovate but wider towards the leaf tip. The ovate-oblong leaf shape (ovalis) is found in the lime (N) variety (Fig. 2a-k).

Leaf tip: A divided leaf tip (retusus) was identified in the varieties of seeded selayar (SB), JC-selayar (JS), selayar-selayar (SS), red pomelo (M), sweet pomelo (G) and batu orange (B). A blunt leaf tip (obtusus) was identified in the varieties of Japanshe citroen (JC), white pomelo (P) and dekopon (D).

A pointed leaf tip (acuminatus) divided (retusus) was found in the varieties of siam (SI) and sweet santang (SM). A sharp-pointed (acutus)-divided (retusus) tip was found in the varieties of lime (N) and a blunt (obtusus)-pointed (acutus)-divided (retusus) tip was identified in the variety of kaffir lime (NN) (Fig. 2a-k).

Leaf base: A blunt leaf base (obtusus) was found in the varieties of seeded selayar (SB), JC-selayar (JS), selayar-selayar (SS), white pomelo (P), batu orange (B), siam orange (SI), sweet santang (SM) and kaffir lime (NN). A pointed leaf base (acutus) was identified in the varieties of Japanshe citroen (JC), red pomelo (M) and dekopon (D). A blunt (obtusus)-rounded (rotundatus) leaf base was found in the variety of sweet pomelo (G) and a rounded (rotundatus) leaf base was identified in the variety of lime (N) (Fig. 2a-k).

Leaf vein arrangement (nervatio/venation): Orange plants have a pinnate leaf vein arrangement (penninervis), which means the leaf has a single main vein that extends from the base to the tip and serves as an extension of the leaf stalk. From the main vein, smaller branching veins emerge sideways, giving the appearance similar to the fins of a fish (Fig. 2a-k).

Leaf margin (margo folii): Crenate leaf margin was found in the varieties seeded selayar (SB), JC-selayar (JS), selayar-selayar (SS), Japanshe citroen (JC), red pomelo (M), batu orange (B), siam orange (SI), dekopon (D), lime (N) and kaffir lime (NN). Sinuate leaf margin was identified in the varieties white pomelo (P), sweet pomelo (G) and sweet santang (SM) (Fig. 2a-k).

Leaf mesophyll (intervenium), color, surface and leaf arrangement on the stem (phyllotaxis): All orange varieties have leaf mesophyll resembling thin paper (papyraceous/chartaceous). The leaf color is dark green with a smooth (laevis) and glossy (nitidus) surface. The leaf arrangement on the stem is alternate (folia sparsa) (Fig. 2a-k).

Width and shape of leaf petiole wings: All orange varieties have leaf petiole wings except for the siam variety (SI). The width of the leaf petiole wings is medium to wide, with obcordate-obdeltate shape in the varieties of red pomelo (M), white pomelo (P) and sweet pomelo (G). They have narrow wings with obdeltate shape in the varieties of seeded selayar (SB), JC-selayar (JS), selayar-selayar (SS), Japanshe citroen (JC), batu orange (B), dekopon (D), lime (N) and kaffir lime (NN). The leaf has narrow to medium wings with obdeltate shapes in the variety of sweet santang (SM) (Fig. 2a-k).



Fig. 1(a-m): Morphological variations of 13 orange plant varieties, (a) Seeded selayar (SB), (b) JC-selayar (JS), (c) Selayar-selayar (SS), (d) Red pomelo (M), (e) While pomelo (P), (f) Sweet pomelo (G), (g) Batu orange (B), (h) Siam orange (SI), (i) Lime (N), (j) Kaffir lime (NN), (k) Dekopon (D), (l) Sweet santang (SM) and (m) Japansche citroen (JC)

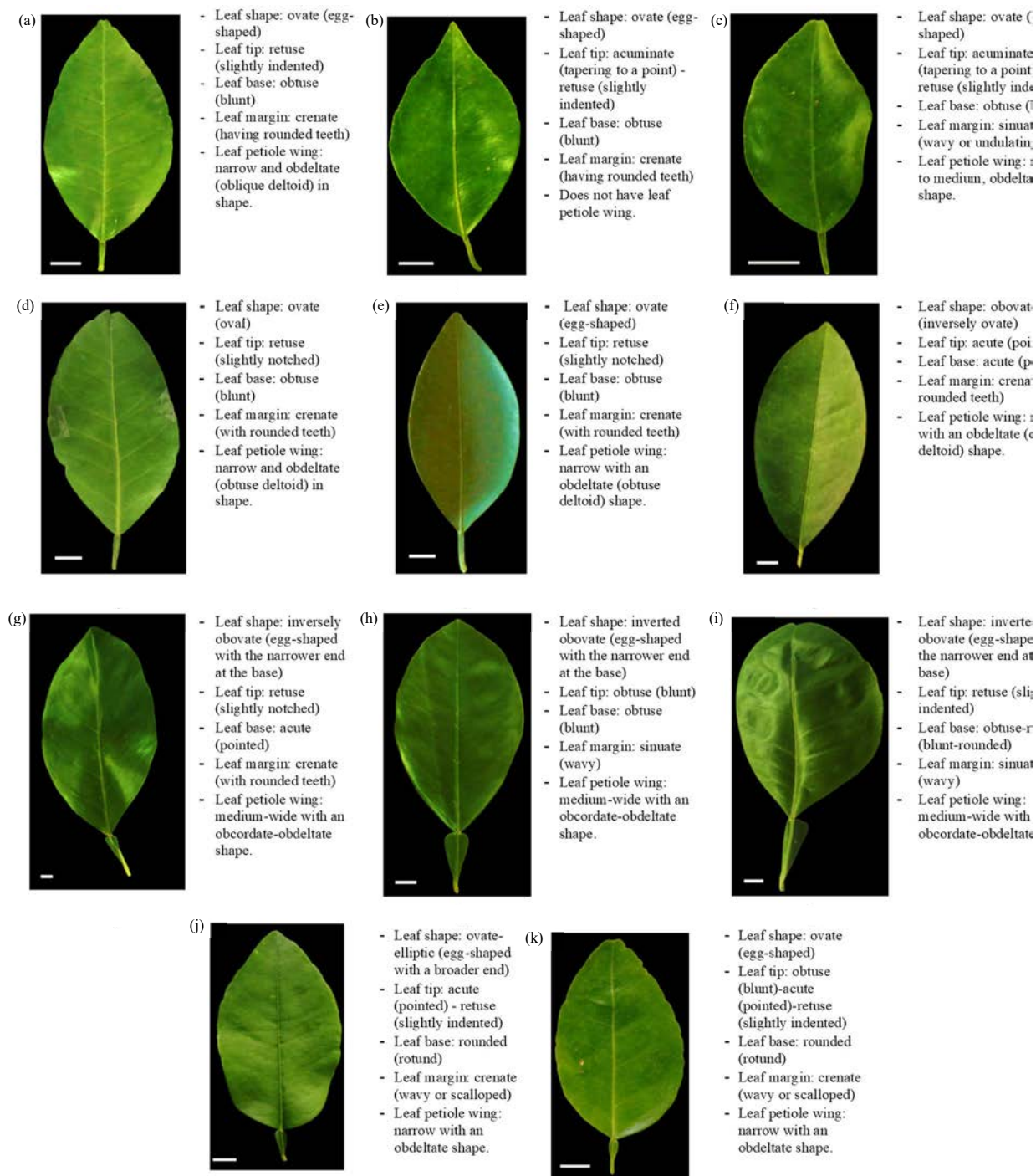


Fig. 2(a-k): Morphological variations of 13 orange plant varieties, (a) Seelayar variety [seeded selayar (SB), JC-selayar (JS) and selayar-selayar (SS)], (b) Siam orange (SI), (c) Sweet santang (SM), (d) Batu orange (B), (e) Dekopon (D), (f) Japansche citroen (JC), (g) Red pomelo (M), (h) White pomelo (P), (i) Sweet pomelo (G), (j) Lime (N) and (k) Kaffir lime (NN)

Tree height and stem diameter: The height of orange plant varieties ranges from 1.75 to 8.83 m, with a diameter ranging from 2.30 to 21.34 cm. The tallest trees are found in seeded selayar (SB), JC-selayar (JS) and selayar-selayar (SS) orange

varieties, reaching approximately 8 m in height with a diameter of around 9 cm. The shortest tree is the sweet santang (SM) tree, measuring 1.75 m in height with a diameter of 2.60 cm (Fig. 1a-m).

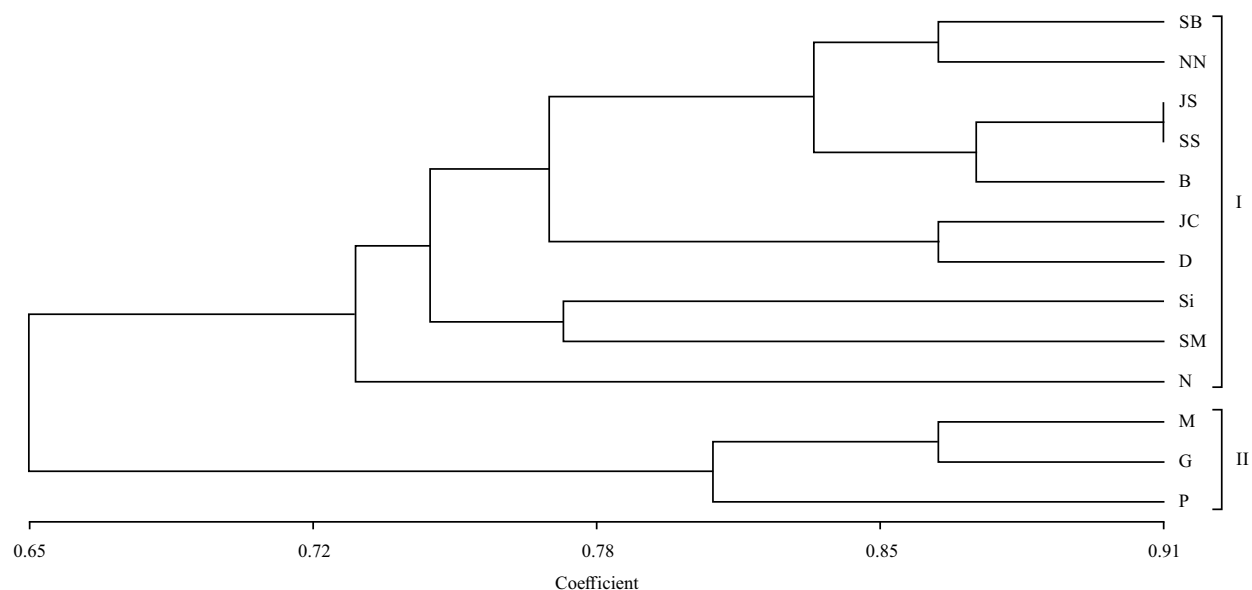


Fig. 3: Dendrogram generated from the cluster analysis of the morphology of 13 orange varieties (Seeded selayar (SB), Kaffir lime (NN), JC-selayar (JS), Selayar-selayar (SS), Batu orange (B), Japansche citroen (JC), Dekopon (D), Siam (SI), Sweet santang (SM), Lime (N), Red pomelo (M), Sweet pomelo (G) and White pomelo (P))

Length, width, thickness and length of leaf stalk: The length of leaves varies among varieties, ranging from 1 to 13.4 cm, with a width of 1.5 to 9.2 cm. The longest leaves are found in red pomelo (M), white pomelo (P) and sweet pomelo (G) varieties, measuring approximately 13 cm in length and around 7.5 cm in width (Fig. 2a-k).

The dendrogram shows two clusters with a similarity coefficient of 65%. Cluster I consists of the varieties seeded selayar (SB), kaffir lime (NN), JC-selayar (JS), selayar-selayar (SS), batu orange (B), Japansche citroen (JC), dekopon (D), siam (SI), sweet santang (SM) and lime (N). On the other hand, Cluster II consists of the varieties red pomelo (M), sweet pomelo (G) and white pomelo (P). Cluster I, with a similarity coefficient of 0.74, is further divided into two sub-clusters, namely sub-cluster 1 and 2. Sub-cluster 1 comprises the varieties seeded selayar (SB), kaffir lime (NN), JC-selayar (JS), selayar-selayar (SS), batu orange (B), Japansche citroen (JC), dekopon (D), siam (SI) and sweet santang (SM). Sub-cluster 2 consists of the variety lime (N). Sub-cluster 1, with a similarity coefficient of 0.75, is further divided into sub-cluster 1.1 and Sub-cluster 1.2. Sub-cluster 1.1 consists of seeded selayar (SB), kaffir lime (NN), JC-selayar (JS), selayar-selayar (SS), batu orange (B), Japansche citroen (JC) and dekopon (D). Sub-cluster 1.2 consists of siam (SI) and sweet santang (SM). Sub-cluster 1.1 and 1.2 have a similarity of 75% (Fig. 3).

The results of the morphological analysis of the 13 orange plant varieties show both similarities and differences in shared characteristics among the varieties. The shared morphological characteristics include the habitus of orange plants, which are generally trees with an upright growth direction (*erectus*), branching on the sympodial stem (main stem difficult to determine), upright branch growth (*fastigiatus*) with a very small angle between the stem and branches, nearly parallel to the main stem. The leaf attachment is *brevipetiolate* (leaf stalk shorter than the leaf blade), the leaf veins are *pinnate* (*penninervis*), the leaf tissue is thin like paper (*papyraceus/chartaceus*), the leaf color is dark green, the leaf surface is smooth (*laevis*) and shiny (*nitidus*) and the leaf arrangement on the stem is scattered with single leaves occupying about one-third (*folia sparsa*). The differing morphological characteristics are observed in the tree and leaf features, such as leaf shape, leaf apex, leaf base, leaf margin, presence of wings on the leaf stalk and width of wings on the leaf stalk.

Leaves are the most diverse vegetative part of plants. Factors contributing to this diversity are adaptations to the environmental conditions in which leaves have evolved and diversified to adapt to various environmental conditions²³. Different plants inhabit different habitats, each with its own unique challenges. Leaves have adapted to various conditions

through the development of different shapes, sizes, structures and surface features that help plants optimize their interactions with light, water and gases such as carbon dioxide and oxygen²⁴. Leaves are primarily responsible for photosynthesis, the process by which plants convert sunlight into chemical energy^{25,26}. The diverse forms and structures of leaves reflect adaptations to maximize photosynthetic efficiency^{27,28}. For example, broad leaves have a larger surface area for light absorption, while needle-shaped leaves of coniferous trees reduce water loss in cold and dry environments²⁹. Leaves also play a crucial role in nutrient acquisition^{30,31}. Different plants have developed specialized leaf structures to acquire nutrients from various sources. In terms of defence mechanisms, leaves have developed various defense mechanisms against herbivore attacks and pathogens^{32,33}. Some leaves have developed thorns or tough textures to deter herbivores, while others produce chemical compounds or toxins that make them unappetizing or toxic to potential threats^{34,35}. For plant variety release, leaf morphology is an important observation component for perennial fruit crops, including orange plants, such as leaf shape, leaf type, leaf characteristics, leaf apex, leaf division, leaf color, leaf type and leaf size.

The differences in morphological characteristics observed in different species are due to their genetic diversity. These genetic differences are not only evident between species but also within a single species, indicating intra-species genetic variability. It is through this genetic diversity that traits within a species vary, known as varieties or even accessions³⁶. The differences in characteristics are also influenced by external factors such as the surrounding environment and the plant's growing location. Morphology is the result of the interaction between genotype and environment. It is used to detect the diversity of plants based on their external structures^{37,38}. The environment is one of the main factors in the growth and development process of plants, leading to the possibility of morphological and physiological differences even among the same plant species³⁹. Environmental factors determine the diversity of a plant population in a specific area, including factors such as elevation, rainfall and humidity^{40,41}.

Based on the cluster analysis of morphological characters shown in Fig. 3 sub-cluster 1.1 consists of seeded selayar (SB), kaffir lime (NN), JC-selayar (JS), batu orange (B), Japansche citroen (JC) and dekopon (D) varieties. This cluster shows a similarity of 75% with sub-cluster 1.2, which consists of siam (SI) and sweet santang (SM) orange varieties. The high similarity value may indicate a close evolutionary relationship or descent from the same ancestors⁴². It may also indicate that both groups share similar characteristics and may belong to

the same group in the context of classification. This indicated that the varieties in these two sub-clusters have many common traits, including stem shape, stem growth direction, branching pattern, branch growth direction, leaf attachment, leaf shape, leaf base, leaf vein arrangement, leaf margin, leaf flesh, leaf color, leaf surface and leaf arrangement.

Anatomical characteristics: Observations of leaf anatomy of the 13 orange plant varieties indicate that stomata are only found on the lower surface (abaxial) of the leaves. All observed varieties have anomocytic stomata type, which means the guard cells are surrounded by a number of certain cells that are not different from other epidermal cells in terms of shape and size. The stomatal guard cells are kidney-shaped and there are trichomes as derivatives of the upper epidermal cells and the distribution of stomata is irregular. Different anatomical characteristics among varieties are found in the size of stomata, stomatal index, upper and lower epidermal cell types and upper and lower epidermal cell walls. The length of the stomata ranges from 12.5-30 µm and the width of the stomata ranges from 7.5-22.5 µm. The stomatal index ranges from 16.07-29.44%, with the lowest stomatal index found in the NN variety (16.07%) and the highest in the batu orange variety (29.44%). The upper epidermal cell type is slightly irregular with 4-6 sides in varieties japansche citroen (JC), sweet pomelo (G), batu orange (B), lime (N), kaffir lime (NN), siam (SI), sweet santang (SM) and dekopon (D) and slightly irregular with 5-6 sides in varieties seeded selayar (SB), Japansche citroen (JS), selayar-selayar (SS), red pomelo (M) and white pomelo (P). The lower epidermal cell type is slightly irregular with 4-6 sides in all varieties except for the variety white pomelo (P), which is slightly irregular with 5-6 sides. The upper epidermal cell walls are slightly undulated-straight in all varieties except for the variety white pomelo (P), which has shallow undulations. The lower epidermal cell walls are slightly undulated-straight in all varieties except for the variety white pomelo (P), which has shallow undulations. The dendrogram results were presented in Fig. 4 show two clusters with a similarity coefficient of 68%. Cluster I consists of selayar keprok varieties, pangkep pomelo and keprok batu. Cluster II consists of Japansche citroen (JC), sweet santang (SM), dekopon (D), siam (SI), lime (N) and kaffir lime (NN) varieties. Cluster II, with a similarity coefficient of 0.79, is further divided into two sub-clusters: Sub-cluster 1 and 2. Sub-cluster 1 consists of Japansche citroen (JC) and sweet santang (SM) varieties, while sub-cluster 2 consists of dekopon (D), siam (SI), lime (N) and kaffir lime (NN) varieties. Sub-clusters 1 and 2 have a similarity of 79% was shown in Fig. 4. The observation results of stomata anatomy indicate that stomata are only found on the lower

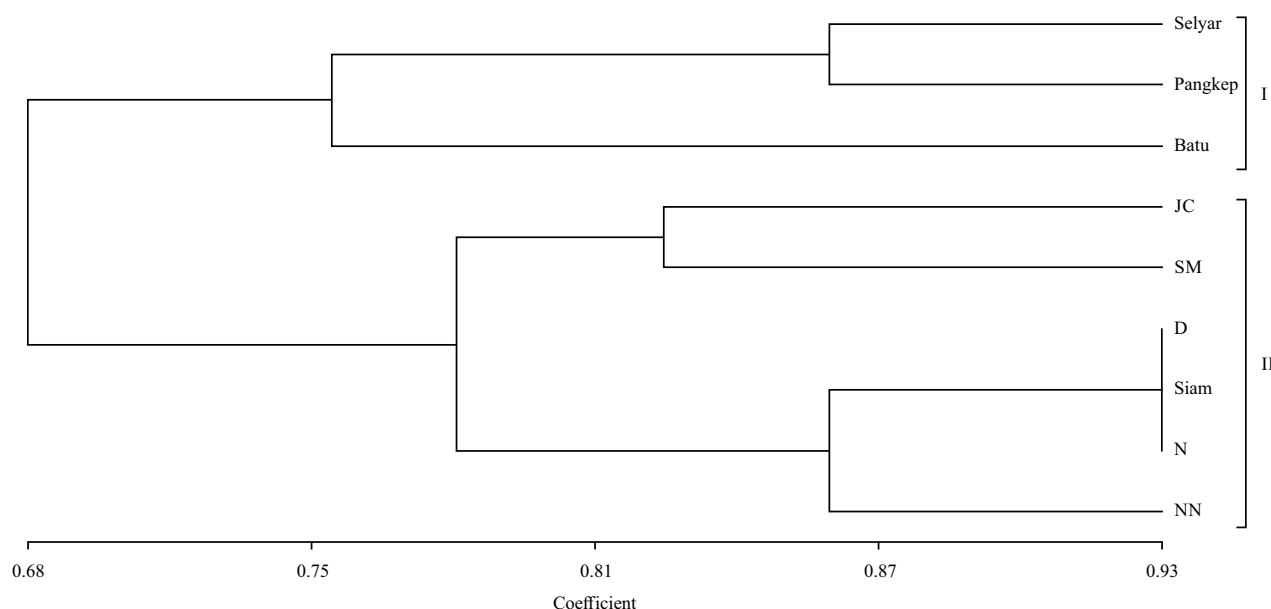


Fig. 4: Dendrogram generated through anatomical cluster analysis of 13 orange varieties

Selayar [seeded selayar (SB), JC-selayar (JS), selayar-selayar (SS)], Pangkep [red pomelo (M), white pomelo (P) and sweet pomelo (G)], batu orange (B), Japanshe citroen (JC), Sweet santang (SM), Dekopon (D), Siam (SI), Lime (N) and Kaffir Lime (NN)

surface (abaxial) of the leaves shown in Fig. 5. The higher density of stomata on the lower leaf surface is an adaptive mechanism of trees to the environment, reducing transpiration⁴³. The lower surface of orange leaves tends to be more protected and shaded compared to the upper surface. Stomata located on the lower surface help reduce excessive evaporation due to sunlight exposure and lower temperature. This assists in maintaining hydration balance in orange plants, especially in dry or hot environmental conditions⁴⁴. The upper surface of orange leaves is susceptible to physical damage, especially when exposed to rain or adverse weather. With the presence of stomata on the lower surface, orange plants can protect stomata from direct contact with raindrops or potential mechanical damage. This helps maintain stomatal integrity and ensures smooth gas exchange⁴⁵. Stomatal index, length and width show variation among orange varieties. The stomatal variation among orange varieties is the result of a combination of genetic factors, environment, physiological adaptation and human selection. Genetic factors play a role, where genetic variation among orange varieties can cause differences in stomatal morphology^{45,46}. Genes involved in the regulation of stomatal number, size and distribution can differ between orange varieties. Differences in the expression of these genes can result in variations in stomatal index, stomatal length and stomatal width⁴⁷. The growing environment can influence stomatal morphology in plants, including orange

varieties. Environmental factors such as temperature, air humidity, light and Carbon dioxide (CO₂) levels can affect stomatal development and size. Orange varieties grown in different environments may show variations in stomatal index, stomatal length and stomatal width. Physiological adaptation comes into play, where stomata play a role in gas exchange, including Carbon dioxide (CO₂) exchange and water vapor transpiration. Variations in stomatal index, stomatal length and stomatal width among orange varieties may result from physiological adaptations to different environmental conditions⁴⁸. Orange varieties grown in dry or humid environments, with different light levels, or with different water requirements may have different stomatal morphologies to optimize gas exchange and hydration balance⁴⁹. Different orange varieties have undergone human selection for centuries to obtain desired traits such as taste, fruit size, disease resistance or productivity. In this selection process, some varieties may have undergone changes in stomatal morphology as a result of desired genetic changes or side effects of selection. This can lead to variations in stomatal index, stomatal length and stomatal width among orange varieties. The results of the diversity analysis of 13 orange varieties showed morphological variability in tree form and leaf shape, while anatomical characteristics showed variability in stomatal size and stomatal index. Similarity analysis revealed that morphological traits formed clusters consisting

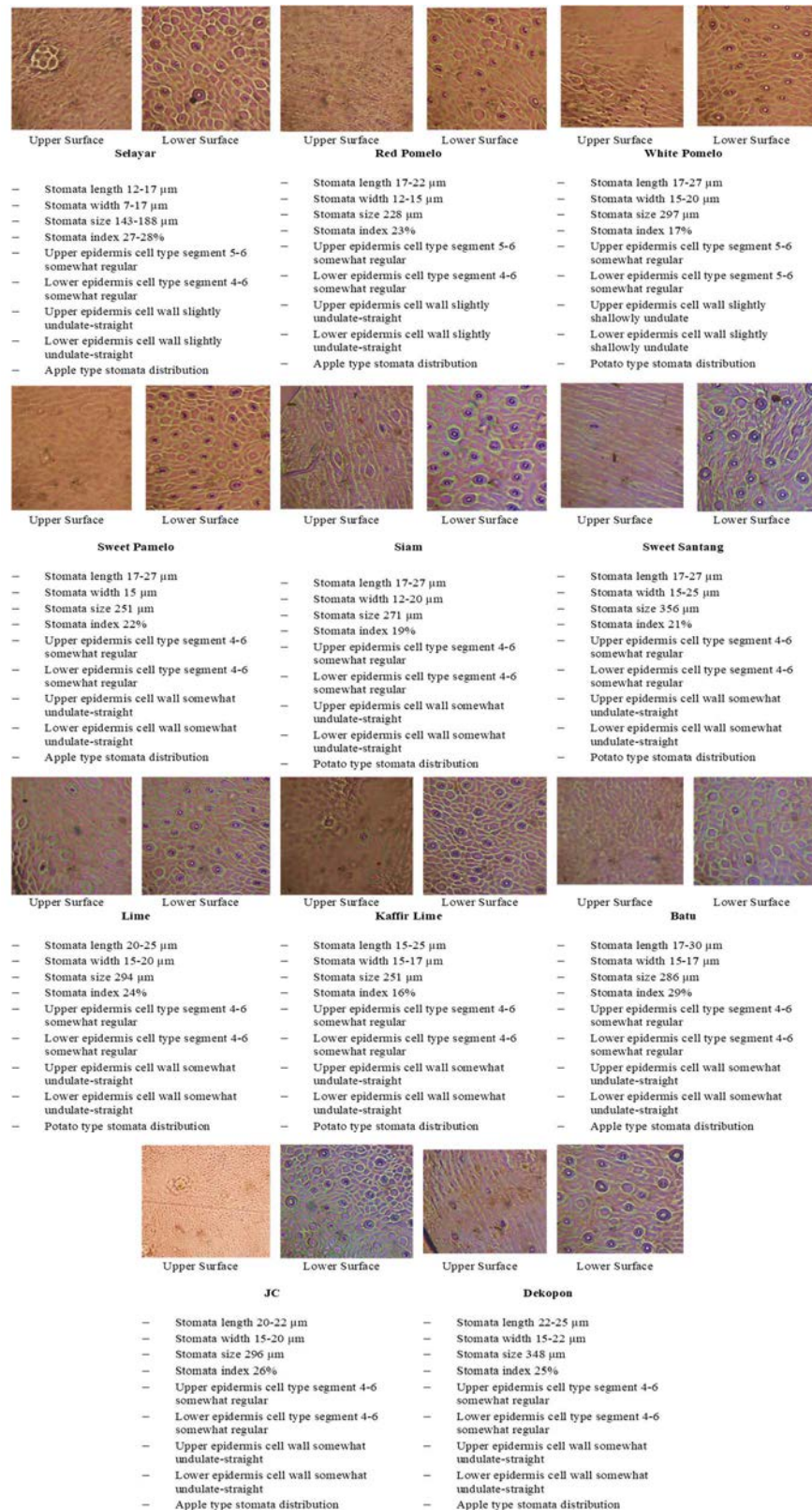


Fig. 5: Anatomical characteristics of leaf stomata of 13 orange varieties in South Sulawesi. Selayar [seeded selayar (SB), JC-selayar (JS), selayar-selayar (SS)], Pangkep [red pomelo (M), white pomelo (P), sweet pomelo (G)], Siam (SI), Sweet santang (SM), Lime (N), Kaffir Lime (NN), batu orange (B), Japansche citroen (JC) and Dekopon (D)

of seeded selayar (SB), kaffir lime (NN), JC-selayar (JS), selayar-selayar (SS), batu (B), Japansche citroen (JC) and dekopon (D) orange varieties with a similarity of 75% to siam (SI) and sweet santang (SM) orange varieties. Meanwhile, anatomical cluster analysis showed that Japansche citroen (JC) and sweet santang (SM) orange varieties had a similarity of 79% to dekopon (D) orange variety. The dendrogram diagram can serve as a basis for determining desired plant traits in plant breeding activities. However, further genetic analysis is needed to strengthen the interpretation and gain a more comprehensive understanding of plant relationships and trait inheritance.

CONCLUSION

The analysis of 13 orange varieties revealed significant variations in both morphological and anatomical traits. Morphological features, such as tree form and leaf shape, showed diversity. Notably, the similarity analysis revealed that morphological characteristics grouped varieties SB, NN, JC-JS, SS, B, JC and D together, sharing a 75% similarity with SI and SM varieties. Concerning anatomical traits, JC and SM orange varieties demonstrated a 79% similarity with D. The resulting dendrogram diagram can be a valuable resource for selecting specific plant traits in future breeding initiatives.

SIGNIFICANCE STATEMENT

In plant breeding efforts, data on the characteristics of the cultivated plants are essential. Plant characterization can be conducted through morphological and anatomical traits. With the availability of this characterization data, it becomes easier to determine the position or relationship among varieties, which can serve as the basis for plant selection. This research aims to identify the diversity of oranges in South Sulawesi based on morphological and anatomical characteristics. The results of this study are expected to serve as a guide in the selection of desired traits in plant breeding.

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