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The phenetic relationship of citrus plants based on the morphological and anatomical characteristics

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Abstract. *Nurzaman M, Setiawati T, Hasan R, Qotrunnada NK, Kusmoro J, Permadi N, Julaeha E. 2024. The phenetic relationship of citrus plants based on the morphological and anatomical characteristics. Biodiversitas 25: 1201-1213.* Indonesia stands as a global biodiversity hub, boasting an exceptional richness that earns it the distinction of being a mega-biodiversity country. Among its diverse ecosystems, the Rutaceae family holds significance, encompassing numerous genera, notably the *Citrus* genus, which showcases a splendid array of citrus plants. This research aimed to determine phenetic relationships based on the morphological and anatomical characters of seven citrus plants (*Citrus reticulata* var. Chokun, *C. reticulata* var. Fremont, *C. reticulata* var. Santang, *C. reticulata* var. Dekopon, *C. aurantium* var. variegata, *C. aurantium*, and *C. limon*) in Parongpong District, West Bandung Regency, West Java. The method used in this research was explorative with purposive sampling. This study collected three samples for respective citrus plants for character observations of leaf morphology, anatomy, and fruit morphology. Morphology and anatomy observations were conducted in the Department of Biology, Padjadjaran University laboratory. The description of morphological and anatomical data obtained from each orange plant was then analyzed using NTSYS PC version 2.11a software to obtain similarity values using the dendrogram analysis method. Phenetic analysis of seven orange plants produced a dendrogram divided into 2 main branches, branch I and branch II, with a similarity coefficient of 0.3846. Branch I consists of Chokun orange (*Citrus reticulata* var. Chokun), Fremont orange (*C. reticulata* cv. Fremont), Santang orange (*C. reticulata* cv. Santang), and variegated orange (*C. sinensis* cv. variegata) and branch II consists of dekopon orange (*C. reticulata* var. Dekopon), Sunkist orange (*C. aurantium*), and lemon (*C. limon*).

Keywords: Anatomy, *Citrus*, morphology, phenetic relationships

INTRODUCTION

Indonesia is a country that has a very high level of biodiversity, meaning that Indonesia is one of the centers of biodiversity in the world, which is called a mega-biodiversity country (UNEP 2018). Indonesia is known as a tropical country that crosses the equator. Tropical climate areas have advantages, including increasing biodiversity. The geographic location and support of high rainfall cause the distribution of flora to be very diverse (Couvreur et al. 2020). One of the biodiversity found in Indonesia is the Rutaceae family. The Rutaceae family consists of various genera, including the *Citrus* genus, which has various types of citrus plants with specific tastes, as referred to in its local name. Citrus plants are fruit plants that originate from Asia and have long been grown in Indonesia, either naturally or cultivated (Sofiyanti et al. 2022a; Kumari et al. 2023). Indonesia has many cultivated citrus plants, from the lowlands to the highlands.

Citrus has a wide variety of types, either in species or in cultivar level of taxa. The citrus varieties most widely cultivated are sweet oranges at 60%, then tangerines (mandarin) at 20%, and the rest are Siamese (tangerine), lemon, kaffir lime, and others (Tuwo et al. 2023). Judging from the extensive distribution of citrus types, several species of citrus possess some cultivars, such as those found in citrus plantations located in Parongpong District, West Bandung Regency, West Java. Most citrus can be consumed directly or through processing with other food ingredients. Citrus is also used as an ornamental plant, and others are used as rootstocks (Sofiyanti et al. 2022a). In addition to becoming a source of nutrition, citrus is also a horticultural commodity, which generates income for the community (Boruah et al. 2023).

Characterization is important in determining biodiversity (Dorice et al. 2020). Cultivars are generally characterized based on regional origin, fruit skin color, fruit flesh color, aroma, and taste. The use of morphological characteristics is a fast method. It is still relatively easy compared to other methods, but the obstacle that arises is the existence of environmental factors that can influence the results of visual characterization (Kim et al. 2022). The phenetic relationship among plants can be determined by observing the similarities in their morphological characteristics (Yuan et al. 2020). An anatomical approach can show correlations between anatomical characters and other characters so that anatomical data can strengthen taxon boundaries, especially for taxonomic evidence such as morphological characteristics that are doubtful (Suratman et al. 2022). Qualitatively, phenetic relationships are determined by comparing the

similarities and differences in characteristics of each taxon, using several character similarities (morphology, anatomy, embryology, palynology, cytology, chemistry, reproductive biology, ecology, and physiology). Relationships between plant species can be analyzed to determine the extent of their dissimilarity by calculating the correlation coefficient, similarity index, taxonomic distance, and group analysis (Yu et al. 2023).

Morphological and anatomical characterization must be done to increase accuracy and confirm the characterization of local citrus plants (Tuwo et al. 2023). Previous studies reported using morphological and anatomical characters in local citrus plants in Poso (Murtando et al. 2016) and Parigi Moutong Regencies (Tuasamu 2018). However, no research has reported the characteristics of citrus plants, especially in Parongpong District, West Bandung Regency. Therefore, it is necessary to identify the diversity of citrus plant types based on morphological and anatomical characteristics to determine their relationships. With the background of the things described above, this research was carried out to determine the morphological and anatomical characteristics of several citrus plants found in Parongpong District, West Bandung Regency, West Java. This research is also expected to obtain information regarding kinship relationships based on the morphological and anatomical characteristics of several types of citrus plants. The study adds a new perspective to the morphological and anatomical characterization in the taxonomy of citrus plants.

MATERIALS AND METHODS

Plant materials

The plant materials used in this research were seven variations of citrus consisting of chokun (*C. reticulata* var. Chokun), dekopon (*C. reticulata* var. Dekopon), fremont (*C. reticulata* var. Fremont), lemons (*C. limon* L.), santang (*C. reticulata* var. Santang), sunkist (*C. sinensis*), and variegata (*C. sinensis* var. variegata). The citrus samples were obtained from the "Has Farm" orange plantation in Parongpong District, West Bandung Regency, West Java, Indonesia.

Research procedure

Exploration

Determination of the sample location was carried out at the "HAS farm" orange plantation located in Parongpong District, West Bandung Regency (S 6^0 5' 52.024", E 107^o 33' 48.002"). The research used a purposive exploration method to determine citrus samples, record the necessary data, and document the exploration results. The site observation results showed that seven variations of citrus were found: chokun, dekopon, fremont, lemon, santang, sunkist, and variegata.

Sampling of citrus leaves and fruit

During field exploration, the sample for leaf morphology, three leaves were taken from the second or third branch from the bottom of the stem. Repetition was carried out three times or three plants. The samples have good morphology and were not defective, so it was easy to identify the morphological characters. The sample was used for fruit observation, taken directly from ripe and harvested trees. Three ripe citrus fruits were taken for each orange plant variation. Three leaves were taken from the second or third stem branch from the bottom to observe leaf anatomy. Repetition was carried out three times. Leave samples of each citrus plant where the leaves are exposed to direct sunlight (not shaded) at around 08.30 AM local time. The samples were put in a ziplock and immediately taken to the laboratory for further observations.

Observation of leaf and fruit morphology

This activity aims to determine leaf and fruit morphology diversity in several observed citrus. This study's leaf morphological characteristics were leaf thickness, leaf surface characteristics, leaf color, leaf shape, maximum leaf length, maximum leaf width, leaf margin shape, leaf base shape, leaf apex shape, leaf vein characteristics, and leaf blade characteristics. The morphological characteristics of the fruit observed in this study were fruit shape, fruit apex shape, fruit base shape, fruit skin color, fruit flesh texture, fruit flesh color, fruit diameter, and fruit length. Characterization refers to the citrus characterization guide issued by IPGRI (1999) and Schmid et al. (2022).

Leaf preparation for stomata observation

The replica method was used to make stomata preparations. The procedure carried out in the laboratory is as follows: (i) The leaves are taken and cleaned from dust or dirt, (ii) The lower surface of the leaves is smeared with transparent nail polish, left for around 5-10 minutes until dry, (iii) The part of the leaf that is smeared with nail polish is covered with clear tape, (iv) Then the clear tape is pulled or taken slowly and carefully and (v) The replica is placed or glued to a glass object and labeled. The preparations were observed using weak to strong magnification for final observation with 400x magnification.

Leaf paradermal observations

This procedure aims to determine the differences in the paradermal structure among the leaves of the observed citrus plants. The leaf paradermal characteristics that were observed included stomata type, number of stomata, stomata density, stomata length and width, the presence of trichomes, and cuticle. The fresh samples of citrus leaves were washed and cleaned to remove dirt and leaf hairs or trichomes. The abaxial of the leaf sample was smeared with transparency nail polish, then waited for about 10 minutes for drying, and then attached with transparency tape evenly. After that, peel off the tape and stick it on the glass object. The number of stomata per millimeter of leaf area, or stomatal density, was measured by counting the number of stomata in the 400x magnification field of view after examining the abaxial leaf impressions under a microscope. The presence of trichomes is categorized as seldom, medium, and congested based on the microscopic observation with 400x magnification.

Description of plants

A description of the plants was then prepared based on the obtained data. An explanation or description was carried out for each type of orange plant based on the results of the research data that had been carried out. The description was carried out to describe the characteristics or characteristics of each orange plant variation.

Phenetic analysis

This step was carried out to obtain similarity values between seven citruses using the dendrogram analysis method. All the characteristics obtained based on the characteristics of leaf morphology, fruit morphology, and leaf anatomy of each type of citrus plant were given code characteristics and transformed into binary data in matrix form. The characteristics were coded from 0 (zero) to the total number of characteristics found. The codes were arranged in tabular form to form a data matrix (Rohlf 1988). The code was created in the table using Microsoft Excel software. This step facilitates data analysis into the NTSYS PC software version 2.11a.

Binary data in the form of Microsoft Excel was then analyzed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method with the help of the Numerical Taxonomy and Multivariate Analysis System (NTSYS) PC version 2.11a software to obtain a dendrogram of relationship for seven citrus. The principle of this analysis is to calculate the level of similarity between types. This software processed The degree of similarity so that it can be seen to what extent the relationship among the citruses being studied was presented in a dendrogram (Rohlf 1988).

RESULTS AND DISCUSSION

Character variations of *Citrus* spp.

The results of exploration at the HAS Farm Citrus Plantation, Parongpong District, West Bandung Regency, found seven-character variations of citrus, including chokun (*C. reticulata* var. Chokun), dekopon (*C. reticulata* var. Dekopon), fremont (*C. reticulata* var. Fremont), santang (*C. reticulata* var. Santang), sunkist (*C. sinensis*), and variegata (*C. sinensis* var. variegata). Those variations are grouped into 3 local citrus names: tangerine, sweet orange, and lemon. Tangerines possess 4 varieties: *Citrus reticulata* var. Chokun, *C. reticulata* var. Fremont, *C. reticulata* var. Santang, *C. reticulata* var. Dekopon. Sweet oranges possess 2 variations: *C. aurantium* var. variegata and *C. aurantium*. While lemon only has 1 variation, *C. limon*. The diversity of citrus plants

Morphological characters of leaf

The leaf morphological characters observed in this study were leaf thickness, leaf surface characteristics, leaf color, leaf shape, maximum leaf length, maximum leaf width, leaf edge shape, leaf base shape, leaf tip shape, leaf vein characteristics, and leaf blade characteristics, as shown at Table 1.

Leaf thickness is one of the internal factors that can influence the rate of evaporation/transpiration (Buckley et al. 2017). The leaf blade's thickness depends on the mesophyll tissue's thickness. The thicker mesophyll layer makes the leaves juicy and tender, and the large number of leaf bones and sclerenchyma fibers make the leaves stiff (Tian et al. 2016). Observations of leaf thickness parameters were measured using a caliper. The results of the research above show that the average thickness of the leaves for each type of citrus plant has varying results. The highest mean leaf thickness was for *C. sinensis* (Sunkist) at 0.43 mm, with a 0.4-0.5 mm range. The lowest leaf thickness in *C. reticulata* (Fremont) and *C. limon* L. (Lemon) was 0.36 mm, with a range of 0.35-0.4 mm. In general, orange leaves seem thick because the cuticle formation on the leaf surface is quite good (Zhu et al. 2018; Cabrera et al. 2023).

Table 1. Comparison of leaf morphological characteristics of *Citrus* spp.

Leaf character	Citrus variations						
	Chokun	**Dekopon**	**Fremont**	**Lemon**	**Santang**	**Sunkist**	**Variegata**
The leaf division	Compound, univariate	Compound, univariate	Compound, univariate	Compound, univariate	Compound, univariate	Compound, univariate	Compound, univariate
Petiole wings	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Leaf thickness (mm)	0.39	0.42	0.36	0.40	0.43	0.43	0.41
Intensity of the green color of the leaf blade	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green
Leaf color variegation	Absent	Absent	Absent	Absent	Absent	Absent	Present
Leaf lamina shape	Ellipse	Ellipse	Ellipse	Ellipse	Ellipse	Ellipse	Ellipse
Leaf lamina length (cm)	10.39	12.47	10.29	13.07	11.48	12.89	10.64
Leaf lamina width (cm)	4.23	6.23	4.33	6.30	5.12	6.12	4.68
Leaf lamina margin	Crenulate	Crenate	Serrate	Serrulate	Serrate	Serrate	Serrate
Angle of leaf base	Obtuse	Obtuse	Obtuse	Obtuse	Obtuse	Obtuse	Obtuse
Leaf apex	Acuminate	Acute	Acuminate	Acuminate	Acuminate	Acute	Acuminate
Nerves on leaf's upper surface	Flat	Protuberant	Flat	Protuberant	Flat	Protuberant	Flat

According to Hoshino et al. (2019), the thickness of the various leaves on each plant can be influenced by the amount of sunlight they receive. The high light intensity can cause leaf cells to be smaller, thylakoids to clump together, and chlorophyll less, resulting in smaller and thicker leaves (Vialet-Chabrand et al. 2017). Leaf flesh is the part of the leaf blade between the leaf's bony system. This part is composed of epidermis and mesophyll tissue. Mesophyll, which consists of parenchyma tissue and functions for photosynthesis, can also function to store water and food reserves. In the mesophyll, the leaf veins and reinforcing tissue are found (McAdam and Brodribb 2018).

In this study, the 7 citrus had varying surface properties, some of which had plain smooth, rough smooth, and dull smooth leaf surfaces. The study shows that chokun, fremont, and lemon have the same leaf surface characteristics: plain smooth. Dekopon and sunkist have rough, smooth leaf surfaces. Meanwhile, santang and variegata have dull, smooth leaf surfaces. This result follows Glime (2017), who found that, in general, the upper surface of orange leaves contains wax and pectin that are smooth and shiny. The orange leaves look shiny because they are covered with cuticles containing a small amount of pectin, so raindrops flow quickly (Opara et al. 2010). Previous research conducted by Adlini and Umaroh (2021) stated that the five variations of citrus (grapefruit, lime, tangerine, kaffir, and green sunkist) also have the same leaf surface, smooth.

All the citrus plant in this study possesses the upper surface of the leaf that was covered by a single layer of epidermis cells. These cells have little or no chloroplasts and secrete a transparent, wax-like substance called cutin that forms a thick cuticle layer. The cuticle reduces water loss from the leaf surface (Kane et al. 2020). In leaves, the epidermis also functions to reduce transpiration. The cuticle protects plants from pest and disease attacks, reduces the rate of transpiration or water evaporation, and reflects sunlight. Cuticles with smooth properties can reduce the attachment and development of spores on the leaf surface so that plants can avoid or minimize disease attacks (Kerstiens 1996; Manasherova 2021).

Generally, the citrus has green leaves but varies in color intensity. Leaf color measurements were conducted on mature leaves of each type of citrus plant. All seven citruses in this study have the same color, dark green (Number 05). The result follows research by Tuasamu (2018), which states that sunkist has dark green leaves. The results of previous research conducted by Hanif et al. (2021) showed that the five types of citrus plants (grapefruit, lime, tangerine, kaffir lime, and green sunkist) also had dark green leaves. Sofiyanti et al. (2022a) also reported that the leaves of lemon are dark green. The leaves on citrus plants have distinct colors between the upper and lower leaves, where the upper leaves tend to be brighter than the surface of the lower leaves (Chan et al. 2021). The color of the leaves is determined by the pigments or substances contained in the cells in the leaves. Plastids contained in leaf cells can contain various pigments, such as green (chlorophyll), yellow (xanthophyll), red (lycopene), or orange (carotene). The color of the leaves depends on the most dominant pigment contained. In general, leaves are green because they have much more chlorophyll than other pigments. Chlorophyll is found in all green plants and is the primary photosynthetic pigment.

The leaves' shape varies on each plant, including citrus. These differences in leaf morphology are often used to identify species (Opara et al. 2010; Suratman et al. 2022). The lamina of the leaves has many variations in size, shape, and color; this variation can be used to identify plants. The study shows that citrus has the same shape, elliptic/oblong. The leaves of tangerines have an elliptical leaf shape. Leaves with elliptic/oblong shape have the widest part of the leaf in the middle of the leaf blade with a ratio of length: width is 1.5 to 2 (Schrader et al. 2021). In this study divided elliptical leave shapes into two groups, expansive ellipse and medium ellipse. The study revealed that dekopon, lemon, and sunkist have the same leaf shape, namely expansive ellipse. Meanwhile, chokun, fremont, santang, and variegata have medium elliptical leaf shapes.

Moreover, the study shows that the highest average leaf length was lemon, 13.07 cm, with a range of 11.7-14.6 cm. Meanwhile, the lowest average leaf length was fremont, 10.29 cm, with a range of 9.2-11.6 cm. Several factors can influence differences in leaf size on each citrus plant. According to Rezai et al. (2006), leaf morphology was influenced by shade or sunlight intensity variations. In the conditions where the plants receive relatively a lot of sunlight without any shade as the condition in the citrus plantation in this study, the plants have leaf length and width larger than plants under the shaded conditions. Leaves are the vegetative organs of plants where photosynthesis occurs and processed. The leaves' shape and size that vary in each plant type can influence photosynthesis activity (Shi et al. 2019).

Therefore, using a ruler, the maximum leaf width is calculated starting from the widest part of the leaf on each type of citrus plant. The study shows that the highest average of leaf width was lemon, 6.30 cm, with a range of 5.3-6.9 cm. Meanwhile, the lowest average leaf width was chokun, 4.23 cm, with a 4-4.4 cm range. The chokun and fremont have average leaf width that were not quite different. The influence of light intensity on physiological processes will influence and be shown in the plant morphological condition that also states the environment was an essential factor in the plant growth process (Feng et al. 2019; Seyedi et al. 2024). This factor can cause similar plants to have differences in the process of gene expression, biochemistry and physiology so that present a relatively different morphologies appearances (Paradiso and Proietti 2022).

The leaf margin on citrus has various characteristics. The study shows that chokun has a crenulate leaf margin. Crenulate is a leaf margin with tiny teeth along the leaf margin. Dekopon has a crenate leaf margin, a leaf margin with rounded teeth along the edge of the leaf. Lemon has serrulate or small serrated leaf margins. Serrulate is a leaf toothed along the margin with small, sharp, forward-facing teeth. Meanwhile, fremont, santang, sunkist, and variegata

have serrate leaf margins, a toothed along the margin, and sharp teeth pointing forward.

Citrus has varying leaf base shapes. The study shows that each orange plant's leaf bases have the same shape, obtuse or blunt. The leaf base measurement angle for each orange citrus in this study was more than 90°, therefore, it has the same category, obtuse. This result followed previous research conducted by Pratiwi and Agustin (2021), which showed that the leaf base of tangerines (*C. reticulata* var. Blanco) possessed an obtuse leaf base.

The leaf apex of citruses has various shapes. The leaf apex of the seven citrus variants in this study was divided into two types, acute or pointed and acuminate or tapered. The citrus variant of dekopon and sunkist possess the acute leaf apex. The acute leaf apex is the state in which the two edges of the leaf meet at the tip to form an acute angle of more than 90° (Sofiyanti et al. 2022a). Meanwhile, chokun, fremont, lemon, santang, and variegata possess the acuminate leaf apex or tapered. The acuminate leaf apex is the state in which two edges of the leaf form an acute angle (<90°) but elongated (Sofiyanti et al. 2022a).

The nerves on leaf's upper surface in the seven citruses are based on the branch bones' arrangement, including the pinnate type (penninerve), where the branch bones are arranged like fins in fish. Based on the seven citrus leaf veins' characteristics, the study revealed protuberant and flat characteristics. Dekopon, lemon, and sunkist have protuberant types of upper leaf nerves. Meanwhile, chokun, fremont, santang, and variegata have leaf flat types of upper leaf nerves.

The leaf division is divided into single leaves (folium simplex) and compound leaves (folium compositum). In this study's seven variations of citrus, all the leaves were included in the compound univariate leaf type, where there is more than one leaf on one petiole. The study shows that dekopon, sunkist, and variegata have the same leaf characteristics, compound univariate with broad leaflets. Lemon and santang have compound univariate with medium leaflets. Meanwhile, chokun and fremont have the characteristic of compound univariate with narrow or small leaflets. Genetic diversity causes differences in morphological characteristics that appear in each different species. Genetic differences occur not only between species; even within one species, there is gene diversity. With the diversity of genes, the characteristics within one species are diverse and varied or are referred to as varieties, even to the point of accession (Kandowangko and Febriyanti 2023).

Fruit morphology

The morphological characteristics of the fruit observed in this study were fruit shape, fruit top shape, fruit base shape, fruit skin color, fruit flesh texture, fruit flesh color, fruit diameter, and fruit length, are shown in Figure 2.

The study on the seven citruses shows a variety of shapes, including spheroid or rounded, pyriform or pear-like, and ellipsoid or elliptical. Chokun, Fremont, and santang have the same fruit shape, spheroid. This follows the research by Adlini and Umaroh (2021), who reported that tangerines generally have a spheroid shape. This characteristic differed from dekopon, which has a pyriform fruit shape, like a pear. According to Andrini et al. (2021), the fruit characteristics of tangerines were spheroid, slightly flattened (obloid), and belly (pyriform). Meanwhile, lemon, sunkist, and variegata have ellipsoid fruit shapes.

The apex and base fruit of the seven citrus in this study showed a variety of types. The fruit apex of chokun, dekopon, fremont, santang, and variegata were truncate. Meanwhile, lemon and sunkist have rounded fruit apex. The fruit bases of chokun, fremont, santang, sunkist, and variegata have the fruit base of truncate type. Dekopon has a fruit base of neck, it has a small conical part at the base of the fruit. The citrus varian of lemon has a convex fruit base shape.

The fruit of the seven citruses showed various colors, and the study shows that chokun, dekopon, and sunkist have orange-green fruit skin color. Fremont has dark orange and green fruit skin, and lemon skin is yellow. This result follows Jentzsch et al. (2022), which reported that the skin of lemons is yellow when the fruit is ripe. Santang has an orange fruit skin color, while variegata has a yellow-green fruit skin color. The fruit of variegata has a unique color compared to the color of oranges in general because it combines yellow and green with an irregular, elongated alternating pattern. During the development of citrus fruit, the color of the fruit, which was originally green, changes to yellow or orange because it is related to the chlorophyll content reduction and increasing carotenoids (Permadi et al. 2021; Nurzaman et al. 2022). At first, the orange fruit is green, but as it ripens, it will change to yellow or orange, which contains many essential oils (Julaeha et al. 2023; Permadi et al. 2023).

Table 2. Comparison of fruit morphological characteristics of *Citrus* spp.

Fruit character	Citrus variations						
	Chokun	**Dekopon**	**Fremont**	**Lemon**	**Santang**	**Sunkist**	**Variegata**
Fruit shape	Rounded	Like a pear	Rounded	Ellipse	Rounded	Ellipse	Ellipse
Fruit apex	Truncate	Truncate	Truncate	Rounded	Truncate	Rounded	Truncate
Fruit base	Truncate	Necked	Truncate	Convex	Truncate	Truncate	Truncate
Fruit skin color	Green-orange	Green-orange	Dark orange and green	Yellow	Orange	Green-orange	Yellow-green
Fruit flesh texture	Fine	Fine	Fine	Fine	Fine	Rough	Rough
Fruit flesh color	Red-orange	Orange	Red-orange	Dark yellow	Orange	Light orange	Light orange
Fruit diameter	5.6 cm	9.06 cm	6.73 cm	7.2 cm	5.8 cm	8.3 cm	6.4 cm
Fruit length	4.8 cm	8.46 cm	5.16 cm	8.2 cm	4.5 cm	8.03 cm	6.37 cm

The texture and color of the fruit flesh of seven citruses in this study can be grouped into smooth and rough textures. Chokun, dekopon, fremont, lemon, and santang have a smooth flesh texture. Meanwhile, sunkist and variegata have a slightly rough flesh texture. Chokun and fremont have an orange-red flesh color. Dekopon and santang have orange flesh. Sunkist and variegata have a light orange flesh color. Meanwhile, lemon has dark yellow flesh.

This study showed that the highest average fruit diameter was dekopon of 9.06 cm with a range of 8.8-9.2 cm. Meanwhile, the lowest average fruit diameter was chokun of 5.6 cm, with a range of 5.3-6.2 cm. The fruit diameter of cokun and santang in this study was only slightly different. The diameter of sunkist fruit in this study was 7.9-9 cm, comparable with previous research that reported the fruit diameter of sweet orange was 7.2-10.3 cm (De Oliveira et al. 2002; Castle and Krezdorn 2022). This study revealed that dekopon was relatively larger than other tangerines. The highest average fruit length was found in dekopon, 8.46 cm, with a range of 8.3-8.8 cm. Meanwhile, the lowest average fruit length was santang, 4.5 cm, with a range of 4.5-4.6 cm. The length of lemon fruit in this study was 8-8.3 cm, which was not much different from the other study, which reported that the length of lemon fruit was around 8-9 cm (Jentzsch et al. 2022).

Leaf paradermal

The leaf anatomical characters observed in this study were stomata type, number of stomata, density, length, width, and trichomes, as shown in Table 3. Variations in citrus morphology were high, as well as in anatomical characteristics such as stomata characteristic (Tuasamu 2018; Sofiyanti et al. 2022b). In this study, stomata were observed on the lower (abaxial) part of the leaves. It revealed that all seven variations of citrus have the same type of stomata, the parasitic type. The parasitic stomata type is a type of stomata in which each guard cell combines with one or more neighboring cells. Its longitudinal axis is parallel to the axis of the neighboring cells and the aperture (Sofiyanti et al. (2022b). This result is in accordance with the research conducted by Tuasamu (2018), the four species of citrus consisting of sweet orange (*C. sinensis*), lime (*C. aurantifolia*), kaffir lime (*C. hystrix* DC), and cui orange (*C. microcarpa*) has the same type of stomata, the parasitic stomata type. Other research was also carried out by Ogundare and Saheed (2012) in Nigeria that types the four species of citrus plants consisted of lemons (*C. limon* L.), gedang limes (*C. paradisi* Macf), tangerines (*C. reticulata*) and sweet orange (*C. sinensis* L.) have the same type of stomata, the parasitic type. Variations in stoma types between plants are a form of plant adaptation to the environment (Lin et al. 2022). The type of stomata can also influence the transpiration process or the entry and exit of gas or water from the environment into the cell (Pautov et al. 2021). Variations in plant leaf stoma types can be caused by internal (genetic traits) and external (habitat or environment) factors (Thompson et al. 2023).

One of the characteristics that can be used as a taxonomic limitation is the number of stomata because the number of each species is different (Zahara and Win 2019). This study showed that the highest average number of stomata was found in lemon, 108.22, with a range of 95-125. Meanwhile, the lowest average number of stomata was found in chokun, 85.89, with a range of 73-94. The greater the number of stomata, the higher the stomata density and the closer the stomata are located to each other (Kosmiatin et al. 2019). In this study, two groups of stomata number were found, a large number of stomata (101-200) and quite a lot (51-100). There were a lot of stomata in lemon (108.22). Meanwhile, quite a lot of stomata are found in chokun (85.89), dekopon (93.89), fremont (99.78), santang (93.44), sunkist (97.22), and variegata (95.11); the number of stomata impacted to the level of stomata density. Differences in the number of stomata per plant are influenced by genetic factors, which are more dominant than environmental factors (Garvita and Wawangningrum 2020). Leaves that grow in dry environments and under high-intensity light tend to have more stomata.

Table 3. Comparison of leaf paradermal characteristics of seven *Citrus* plants

Leaf anatomy	Citrus variations						
	Chokun	**Dekopon**	**Fremont**	**Lemon**	**Santang**	**Sunkist**	**Variegata**
Stomata type	Parasitic	Parasitic	Parasitic	Parasitic	Parasitic	Parasitic	Parasitic
Number of stomata	85.89	93.89	99.78	108.22	93.44	97.22	95.11
Stomata density	437.65/mm^2	478.41/mm^2	508.42/mm^2	551.45/mm^2	476.15/mm^2	495.4/mm^2	484.64/mm^2
Stomata length	18.37 µm	18.94 µm	21.40 µm	19.27 µm	17.92 µm	20.34 µm	18.68 µm
Stomata width	13.99 µm	13.27 µm	15.94 µm	14.88 µm	14.02 µm	15.49 µm	13.29 µm
Stomata length	18.37µm	18.94 µm	21.4 µm	19.27 µm	17.92 µm	20.34 µm	18.68 µm
The presence of trichomes	Seldom	Medium	Medium	Seldom	Congested	Medium	Seldom

Stomata density is the number of stomata contained in a certain unit area. When observing under a microscope, the number of stomata per unit area of the field of view is counted and converted into mm2 area units (Thompson et al. 2023). The research showed that the highest average stomata density in lemon was 551.45/mm^{2,} with a range of 499.36-636.94/ mm^2. Meanwhile, the lowest mean stomata density was found in chokun, 437.65/mm^2 with a range of 371.97-478.98/mm^2. Varying stomata density can be caused by environmental and genetic factors (Pautov et al. 2021). High and low levels of stomata density are classified into 3 parts: low density (<300/mm^2), medium density (300-500/mm^2) and high density (>500/mm^2) (Qi et al. 2017). This study revealed stomata density was divided into 2 groups: high stomata density (>500/mm^2) and medium stomata density (300-500/mm^2). Stomata density is high in Fremont oranges (508.42/mm^2) and lemons (551.45/mm^2), while the stomata density is medium in chokun oranges (437.65/ mm^2), dekopon (478.41/mm^2), santang (476.15/ mm^2), sunkist (495.4/ mm^2) and variegata (484.64/ mm^2). The stomata density of lemon in this study was almost the same as previous research conducted by Sofiyanti et al. (2022b) reported that the stomata density of lemon was 541.45/mm^2 (high stomata density).

The study showed that the highest average stomata length was found in fremont, 21.4 µm, with a range of 18.93-23.33 µm, while the lowest average stomata length was found in santang, 17.92 µm with a range of 17.91-18.25 µm. Stomata length is classified into 3 sizes: (i) Less long if the length of the stomata is <20 µm; (ii) Length if it has a length of 20-25 µm; and (iii) Very long size if the stomata length is >25 µm (Kosmiatin et al. 2019). In this study, stomata length was catagerized into less long (<20 µm) and long (20-25 µm). Stomata size were less long in chokun (18.37µm), dekopon (18.94 µm), lemon (19.27 µm), santang (17.92 µm) and variegata (18.68 µm). Meanwhile, long stomata were found in fremont (21.4 µm) and sunkist (20.34 µm). The lemon stomata length in this study was 19.27 µm, almost the same as the results of previous research conducted by Sofiyanti et al. (2022b) that recorded the length of the stomata in lemon was 19 µm.

Several factors, such as genetics and environmental factors, can cause variations in length and width of stomata. According to Purnama et al. (2021), the size and density of stomata are related to resistance to water stress, whereas plants considered resistant to drought stress will have a lower stomata density and be reduced in length and width. The research shows that the highest average stomata width in fremont was 15.94 µm, with a range of 14.09-18.45 µm. Meanwhile, the lowest average stomata width was found in dekopon, 13.27 µm, with a range of 12.35-15.40 µm. According to Shtein et al. (2017), differences in the number and size of stomata are caused by genetics and the environment. Width sizes are grouped into three: less wide sizes (<19.42 µm), wide sizes (19.42-38.84 µm), and very wide sizes (>38.84 µm). In this study, the size of the stomata width in the 7 variations of orange plants was in the same category, less wide (<19.42 µm). In previous research conducted by Sofiyanti et al. (2022b), the 5 types of citrus plants (lime, kaffir lime, lemon, musk, and lime) also have the same average size of stomata width, less wide (<19.42 µm).

Trichomes are one of the epidermal derivatives outermost to the other derivatives (Bashir et al. 2020). Trichomes play a role in supporting plant physiological activities and as important morphological and anatomical parameters in plant resistance (Rahman et al. 2022). This study uses the leaves' upper (adaxial) surface using a Dino-Lite Digital Microscope with a magnification of ±50x to observe the presence of trichomes. The observations showed trichomes on each leaf of each orange plant in all seven variations of orange plants. The presence of trichomes in this study was divided into three groups: seldom, medium, and dense trichomes. In chokun, lemon, and variegata were categorized into the seldom presence of trichomes. This study revealed that in dekopon, fremont, and sunkist, trichomes were medium or moderate; meanwhile, santang had dense trichomes.

Description of *Citrus spp*.

*Tangerine (*Citrus reticulata *Blanco var. Chokun)*

Leaf thickness is 0.39 mm; the leaf surface is smooth and plain; the leaf color is dark green; the leaf shape is medium elliptical; the maximum leaf length is 10.39 cm; maximum leaf width 4.23 cm; the shape of the edges is crenulate/small with a tip, the tip of the leaf is acuminate/ tapered, the shape of the base of the leaf is obtuse/obtuse, the nature of the leaf veins is unclear, the nature of the compound leaf blade is narrow/small, and the leaf area is 52 cm^2. The shape of the fruit is spheroid/rounded, the top shape of the fruit is truncate/flat, the base shape of the fruit is truncate/flat, the color of the fruit skin is orange green, the texture of the fruit flesh is smooth, the color of the fruit flesh is red-orange, the fruit diameter is 5.6 cm, and the length of the fruit is 4.8 cm. Parasitic stomata type, number of stomata 85.89 (quite a lot), stomata density 437.65/mm^2 (medium stomata density), stomata length 18.37 µm (not long enough), stomata width 13.99 µm (not wide enough) and presence trichomes were seldom.

*Tangerine (*Citrus reticulata *Blanco var. Dekopon)*

Leaf thickness is 0.42 mm; the leaf surface is smooth and rough; the leaf color is dark green; the leaf shape is wide elliptical; the maximum leaf length is 12.47 cm; maximum leaf width 6.23 cm; the shape of the edges is crenate, the tip of the leaf is acute, the shape of the base of the leaf is obtuse, the leaf veins are clear, the leaf blades are compound, the leaflets are wide and the leaf area is 93 cm^2. The shape of the fruit is pyriform/ like a pear; the top shape of the fruit is truncate/ flat; the basic shape of the fruit is collared with neck/necked; the color of the skin is green-orange; the texture of the fruit is smooth, the color of the flesh is orange, the diameter of the fruit is 9.06 cm, and the length of the fruit is 8.46 cm. Parasitic stomata type, number of stomata 93.89 (quite a lot), stomata density 478.41/mm^2 (medium stomata density), stomata length 18.94 µm (not long enough), stomata width 13.27 µm (not wide enough) and presence medium trichomes.

*Tangerine (*Citrus reticulata *Blanco var. Fremont)*

Leaf thickness is 0.36 mm; the leaf surface is smooth and plain; the leaf color is dark green; the leaf shape is medium elliptical; the maximum leaf length is 10.29 cm; the maximum leaf width 4.33 cm; the shape of the edges is serrate/large serrated, the tip of the leaf is acuminate/ tapered, the shape of the base of the leaf is obtuse/obtuse, the nature of the leaf veins is unclear, the nature of the compound leaf blade is narrow/small, and the leaf area is 59.85 cm^2. The shape of the fruit is spheroid/rounded, the top shape of the fruit is truncate/flat, the base shape of the fruit is truncate/flat, the color of the fruit skin is dark orange and green, the texture of the fruit flesh is smooth, the color of the fruit flesh is red-orange, the fruit diameter is 6.73 cm, and the length of the fruit is 5.16 cm. Parasitic stomata type, number of stomata 99.78 (quite a lot), stomata density 508.42/mm^2 (high stomata density), stomata length 21.4 µm (long), stomata width 15.94 µm (less wide) and the presence of trichomes currently.

*Lemon (*Citrus limon *(L.) Burm.f)*

Leaf thickness is 0.36 mm; smooth smooth leaf surface, dark green leaf color, wide elliptical leaf shape, maximum leaf length 13.07 cm; maximum leaf width 6.3 cm; the shape of the edges is serrulate/small serrated, the tip of the leaf is acuminate/tapered, the shape of the base of the leaf is obtuse/obtuse, the nature of the leaf veins is clear, the nature of the leaf blade is medium compound and the leaf area is 103.18 cm^2. The shape of the fruit is ellipsoid, the shape of the top of the fruit is rounded, the base shape of the fruit is convex, the skin color is yellow, the texture of the fruit flesh is smooth, the color of the fruit flesh is dark yellow, the fruit diameter is 7.2 cm, and the length of the fruit is 8.2 cm. Parasitic stomata type, number of stomata 108.22 (many), stomata density 551.45/mm^2 (high stomata density), stomata length 19.27 µm (not long enough), stomata width 14.88 µm (not wide enough) and the presence of trichomes seldom.

*Tangerine (*Citrus reticulata *Blanco var. Santang)*

Leaf thickness is 0.4 mm; the leaf surface is smooth and dull; the leaf color is dark green; the leaf shape is medium elliptical; the maximum leaf length is 11.48 cm; the maximum leaf width 5.12 cm; the shape of the edges is serrate/large serrated, the tip of the leaf is acuminate/ tapered, the shape of the base of the leaf is obtuse/obtuse, the nature of the leaf veins is unclear, the nature of the compound leaf blade is medium and the leaf area is 69.9 cm^2. The shape of the fruit is spheroid/rounded, the top shape of the fruit is truncate/flat, the base shape of the fruit is truncate/flat, the skin color is orange, the flesh texture is smooth, the flesh color is orange, the fruit diameter is 5.8 cm, and the fruit length is 4.5 cm. Parasitic stomata type, number of stomata 93.44 (quite a lot), stomata density 476.15/mm^2 (medium stomata density), stomata length 17.92 µm (not long enough), stomata width 14.02 µm (not wide enough) and presence dense trichomes.

*Sunkist (*Citrus sinensis *(L.) Osbeck)*

Leaf thickness is 0.43 mm; the leaf surface is smooth and rough; the leaf color is dark green; the leaf shape is wide elliptical; the maximum leaf length is 12.89 cm; the maximum leaf width 6.12 cm; the shape of the edges is serrate/large serrated, the leaf tip is acute/pointed, the shape of the base of the leaf is obtuse/obtuse, the nature of the leaf veins is clear, the nature of the compound leaf blade is wide and the leaf area is 90 cm^2. The shape of the fruit is ellipsoid, the top of the fruit is rounded, the base of the fruit is truncate, the skin color is green-orange, the flesh texture is slightly rough, the flesh color is light orange, the diameter of the fruit is 8.3 cm and the length of the fruit is 8.03 cm. Parasitic stomata type, number of stomata 97.22 (quite a lot), stomata density 495.4/mm^2 (medium stomata density), stomata length 20.34 µm (long), stomata width 15.49 µm (less wide) and the presence of trichomes currently.

*Sweet orange (*Citrus sinensis *(L.) Osbeck var.Variegata)*

Leaf thickness is 0.41 mm; the leaf surface is smooth and dull; the leaf color is dark green; the leaf shape is medium elliptical; the maximum leaf length is 10.64 cm; the maximum leaf width 4.68 cm; the shape of the edges is serrate/large serrated, the tip of the leaf is acuminate/ tapered, the shape of the base of the leaf is obtuse/obtuse, the nature of the leaf veins is unclear, the nature of the compound leaf blade is wide and the leaf area is 56 cm^2. The shape of the fruit is ellipsoid, the top of the fruit is rounded, the base shape of the fruit is truncate, the skin color is yellow-green, the flesh texture is slightly rough, the flesh color is light orange, the diameter of the fruit is 6.4 cm, and the length of the fruit is 6.37 cm. Parasitic stomata type, number of stomata 95.11 (quite a lot), stomata density 484.64/mm^2 (medium stomata density), stomata length 18.68 µm (not long enough), stomata width 13.29 µm (not wide enough) and presence trichomes were seldom.

Phenetic analysis

All the characteristics that have been obtained based on the characteristics of leaf morphology, fruit morphology and leaf anatomy of each type of citrus plant are given code characteristics. After being given a code description of the characteristics and characteristics, it is then arranged into a data matrix to make data analysis easier. Data analysis was carried out using the Numerical Taxonomy and Multivariate Analysis System (NTSYS) PC version 2.11a software program based on 12 leaf morphological characteristics, 8 fruit morphological characteristics, and 6 leaf anatomical characteristics from the 7 variations of citrus plants studied.

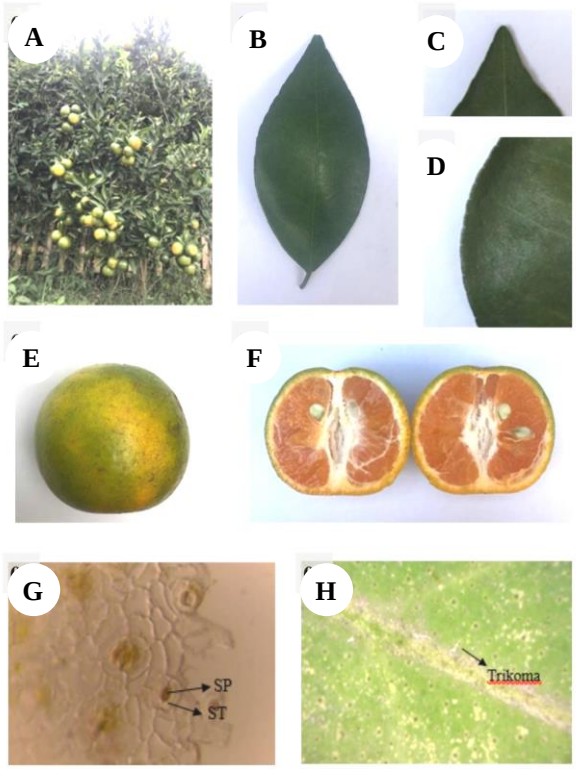

Figure 1. Tangerine leaves (*Citrus reticulata* Blanco var. Chokun). A. Tree, B. Leaves, C. Acuminate/tapered leaf tips, D. Edges crenulate leaves, E. Fruit, F. Color of flesh, G. Type of parasitic stomata, H. Seldom presence of trichomes

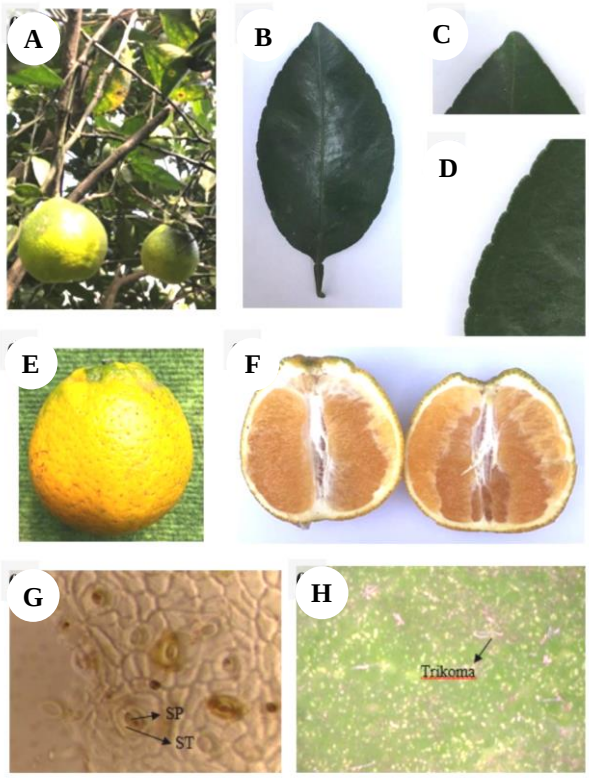

Figure 2. Tangerine (*Citrus reticulata* Blanco var. Dekopon). A. Tree, B. Leaves, C. Acute/pointed leaf tips, D. Crenate leaf margins, E. Fruit, F. Fruit flesh color, G. Parasitic stomata type, H. Moderate presence of trichomes

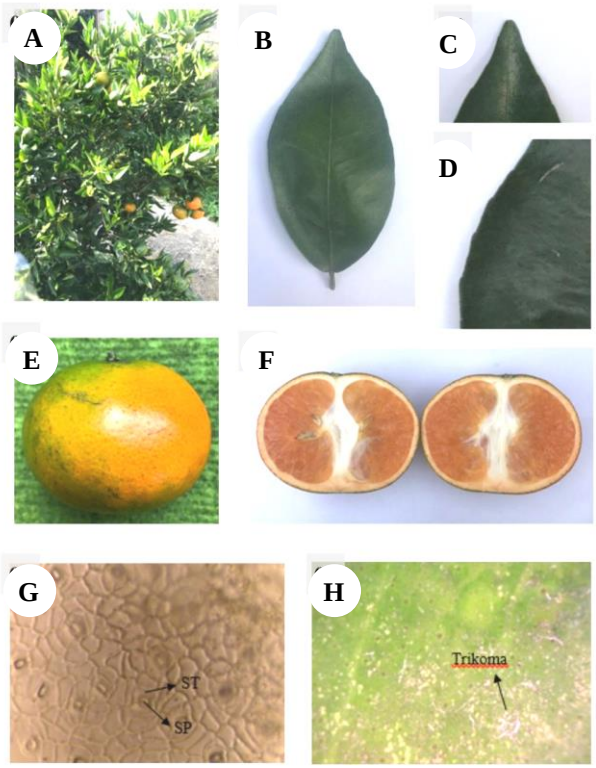

Figure 3. Tangerine (*Citrus reticulata* Blanco var. Fremont). A. Tree, B. Leaves, C. Acuminate/tapered leaf tips, D. Large serrate/serrated leaf margins, E. Fruit, F. Fruit flesh color, G. Parasitic stomata type, H. Moderate presence of trichomes

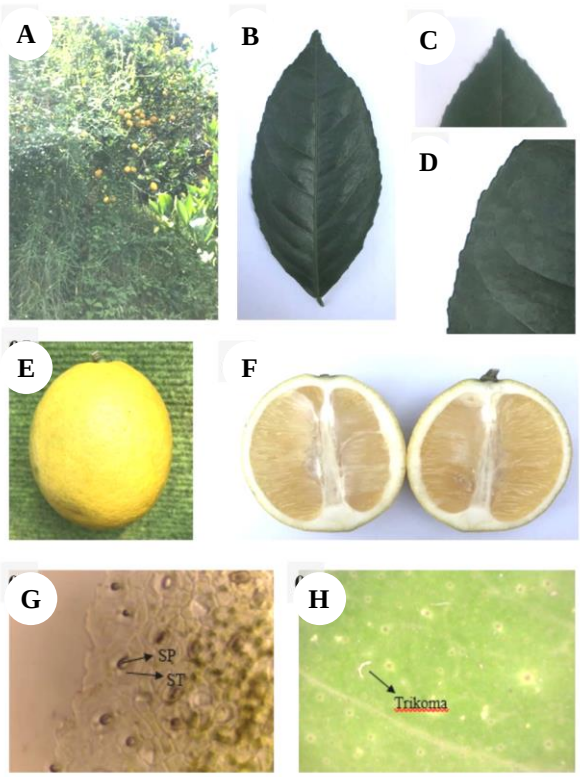

Figure 4. Lemon (*Citrus limon* (L.) Burm.f). A. Tree, B. Leaves, C. Acuminate leaf tips/ tapered, D. Serrulate/small serrated leaf margins, E. Fruit, F. Color of flesh, G. Type of parasitic stomata, G. The presence of trichomes are seldom

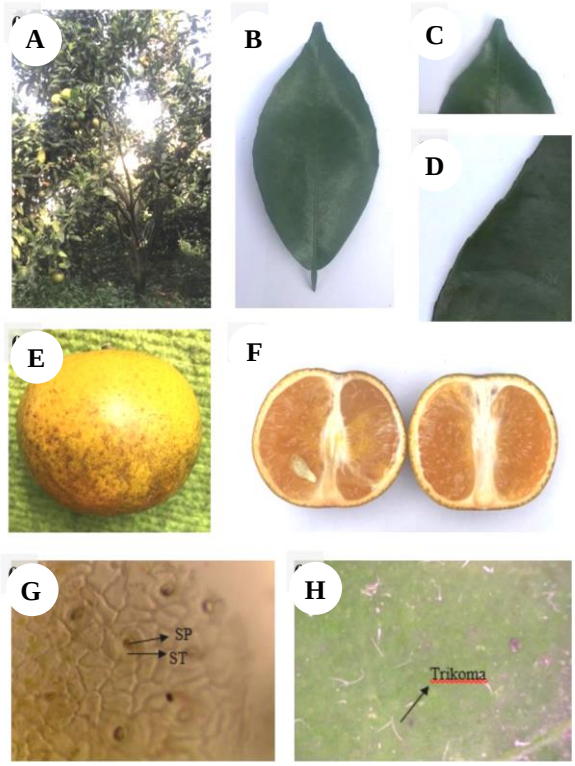

Figure 5. Tangerine (*Citrus reticulata* Blanco var. Santang). A. Tree (var. Santang), B. Leaves, C. Acuminate/tapered leaf tips, D. Serrate/large serrated leaf margins, E. Fruit, F. fruit flesh color, G. Type of parasitic stomata, H. Presence of dense trichomes

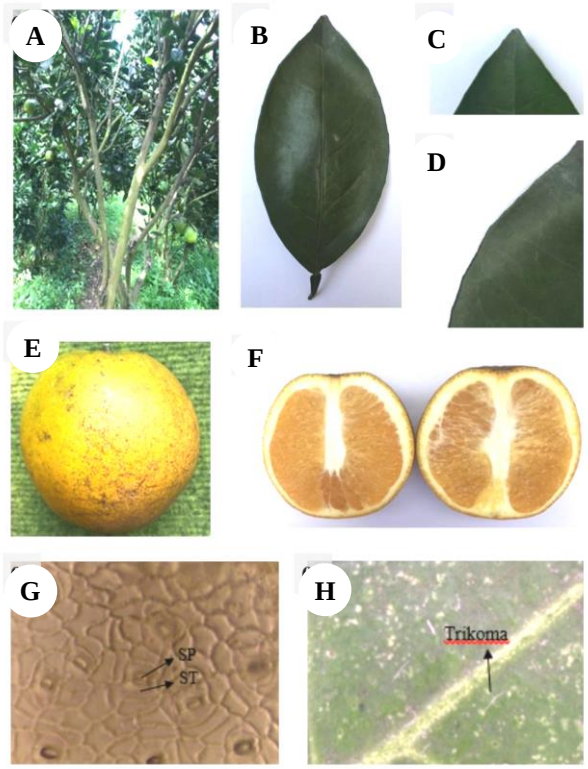

Figure 6. Sunkist Orange (*Citrus sinensis* (L.) Osbeck). A. Tree, B. Leaves, C. Acute/pointed leaf tips, D. Leaf edges serrate/large serrated, E. Fruit, F. Color of flesh fruit, G. Type of parasitic stomata, H. Presence of moderate trichomes

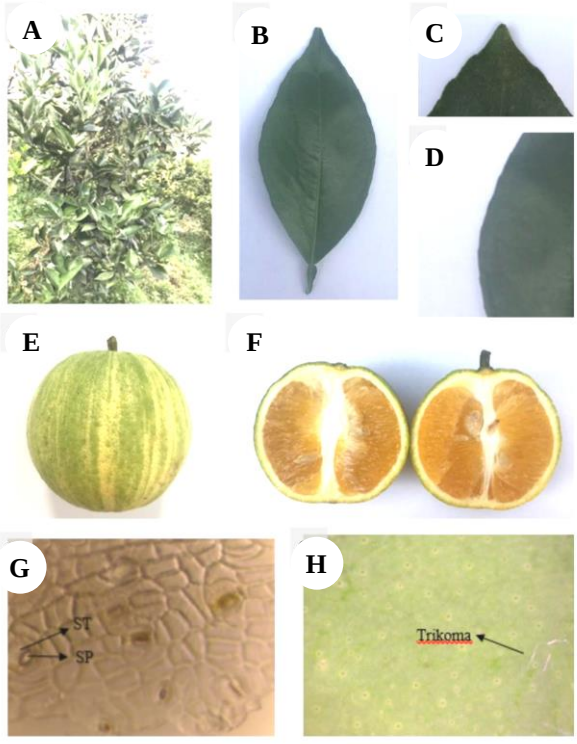

Figure 7. Sweet Orange (*Citrus sinensis* (L.) Osbeck) (var. Variegata). A. Tree, B. Leaves, C. Acuminate leaf tips/tapered, D. Serrate leaf margins/large serrated, E. Fruit, F. Color of flesh, G. Type of parasitic stomata, H. The presence of trichomes are seldom.

The phenetic analysis produces a dendrogram that divides the seven citruses variations into two main branches, branch I and branch II. Branch I consists of chokun, fremont, santang, and variegata. Branch II consists of dekopon, sunkist, and lemon. Branches I and II have a similarity index of 0.3846, which is separated by characteristics 6 (leaf edge shape), 15 (fruit skin color), 17 (fruit flesh color), and 25 (presence of trichomes). Branch I consists of 2 subbranches, subbranch I and subbranch II. Sub-branch I consists of 2 groups, group I and group II, where the group I consists of chokun and fremont with a similarity index value of 0.7308 separately because they have differences in characteristic 6 (leaf margin), 11 (leaf area), 15 (fruit skin color), 18 (fruit diameter), 22 (stomata density), 23 (stomata length) and 25 (presence of trichomes). Group II only consists of santang. The similarity index between group I and group II is 0.6154 because they have differences in characteristics 1 (leaf surface properties), 4 (maximum leaf length), 5 (maximum leaf width), 10 (characteristics of leaf blades), 15 (fruit skin color), 17 (fruit flesh color) and 25 (presence of trichomes). Subbranch II only consists of variegata. The similarity index between subbranch I and subbranch II is 0.5385, which is separated because of characteristics 0 (leaf thickness), 10 (leaf blade characteristics), 12 (fruit shape), 15 (color fruit skin), 16 (flesh texture), 17 (flesh color) and 19 (fruit length).

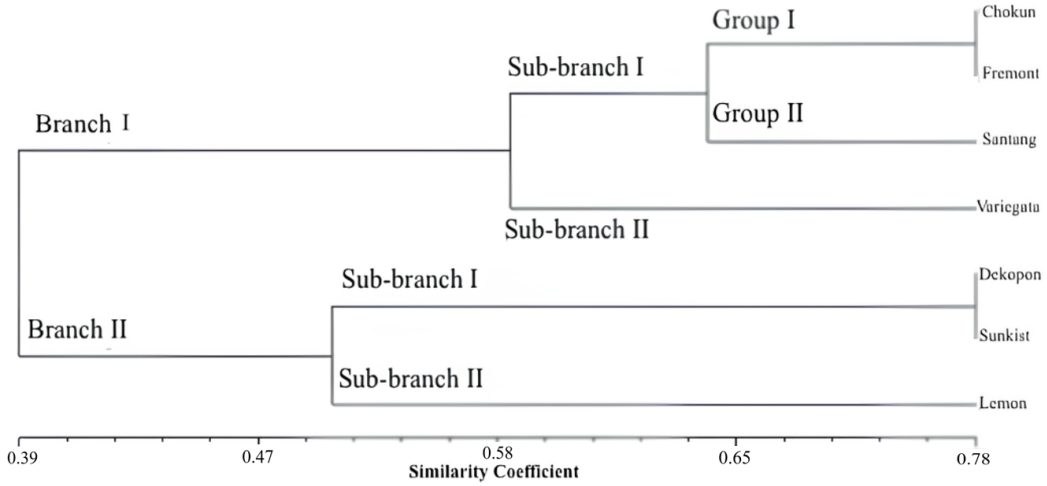

Figure 8. Dendrogram of grouping types of *Citrus* spp. based on morphological and anatomical characteristics

Branch II consists of 2 subbranches, subbranch I and subbranch II. Subbranch I consist of *C. reticulata* (Dekopon) and *C. sinensis* (Sunkist) with a similarity index value of 0.7308, which are separated because of characteristics 6 (leaf margin shape), 12 (fruit shape), 13 (fruit top shape), 14 (basic shape of the fruit), 16 (flesh texture), 17 (flesh color) and 23 (stomata length). Sub-branch II only consists of lemon. The similarity index between sub-branch I and sub-branch II is 0.5 apart because of characteristics 0 (leaf thickness), 1 (leaf surface characteristics), 6 (fringe shape), 8 (leaf tip shape), 10 (leaf blade characteristics), 14 (fruit base shape), 15 (fruit skin color), 17 (fruit flesh color), 21 (number of stomata), 22 (stomata density) and 25 (trichome density). The orange variation that most closely related are chokun and fremont, as well as dekopon and sunkist with a similarity value of 0.7308. According to Kandowangko and Febriyanti (2023), a similarity value closer to 1 indicates that the level of kinship is closer, whereas if the similarity value is closer to 0, the level of kinship is increasingly distant.

The dendrogram results above show that of the 7 variations of orange plants. There are 2 main branches, branch I and branch II, with a similarity coefficient of 0.3846. According to Habiba et al. (2019), the similarity level is far if it is less than 0.6 or 60%. Species with many similarities will have closer kinship relationships; conversely, species with fewer similarities will have more distant kinship relationships (Muntoreanu et al. 2011). Previous research on the diversity of citrus varieties in the Tomini Bay coastal region of Gorontalo revealed that citrus has distinct and varied morphological characteristics that were impacted by environmental and genetic variables. That research found the value of diversity index was 0.8103, that was considered low. It was also recorded a similarity coefficient of 64.3% in the group consisted of seven citruses on the dendrogram, and formed two primary groups that were classified into smaller categories at the species diversity level (Kandowangko and Febriyanti 2023). Morphological characterization of the same citrus variation in this study is relatively comparable with other locations in Indonesia (Murtando et al. 2016; Sofiyanti et al. 2022). However, it also has some differences with another citrus in some characteristics, especially the fruit size, such as in Parang Island Karimunjawa (Hermin et al. 2018). The diversity of citrus morphology can be caused by differences in ecological factors such as temperature, water availability, and light intensity in the habitat where citrus is found in Indonesia (Traband et al. 2023).

In conclusion, this studi showed that the morphological characteristics of leaves and fruit that can be used to differentiate citruses are leaf thickness, leaf surface characteristics, leaf shape, maximum leaf length, maximum leaf width, leaf edge shape, and leaf tip shape. The characteristics of leaf veins, leaf blades, leaf area, fruit shape, fruit base shape, fruit top shape, fruit skin color, fruit flesh texture, fruit flesh color, fruit diameter, and fruit length were the main parameters. Leaf paradermal characteristics that can differentiate citrus are the number of stomata, density, length, width, and trichomes presence. Phenetic analysis of seven variations of citruses produced a dendrogram divided into two main branches, branch I and branch II, with a similarity coefficient of 0.3846. Branch I consists of chokun (*C. reticulata* var. Chokun), fremont (*C. reticulata* var. Fremont), santang (*C. reticulata* var. Santang) and variegata (*C. aurantium* var. variegata), dekopon (*C. reticulata* var. Dekopon), sunkist (*C. sinensis*), and lemons (*C. limon* L.). The results of this study contribute to the knowledge of citrus diversity in understanding the phenetic relationships that help classify citrus species more accurately, provide insights in analyzing morphological traits, researchers can identify superior cultivars with desirable characteristics.

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