BIOTROPIA ISSN: 0215-6334 | e-ISSN: 1907-770X
The Southeast Asian Journal of Tropical Biology
Vol. 31 No. 2, 2024: 157 - 168
DOI: 10.11598/btb.2024.31.2.1872

Research Article

DIVERSITY OF *Citrus* spp. FROM NORTH PENAJAM PASER REGENCY (IKN), EAST KALIMANTAN, BASED ON MORPHOLOGICAL CHARACTERS

Linda Oktavianingsih[1]*, Noviana Crisdayanti[2], Muhammad Fauzi Arif[3], And Medi Hendra[4]

[1]Laboratory of Animal Anatomy and Microtechnique, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75119, Indonesia. [2]Undergraduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75119, Indonesia. [3]Laboratory of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75119, Indonesia. [4]Laboratory of Plant Anatomy and Systematics, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75119, Indonesia

ARTICLE HIGLIGHTS

- This research is the first study to provide data on the diversity of *Citrus* spp. from North Penajam Paser Regency (IKN) area, East Kalimantan for further development and breeding program.
- This research offers new information on the distribution of *Citrus* spp. from the North Penajam Paser Regency (IKN) area, East Kalimantan.
- This research provides information on the phenetic relationships among *Citrus* spp. from the North Penajam Paser Regency (IKN) area, East Kalimantan, and the morphological characteristics that play the most significant role in their clustering.

Article Information

Received 14 February 2023
Revised 17 November 2023
Accepted 15 March 2024

***Corresponding author, e-mail:**
oktavianingsihlinda@yahoo.com,
lindaoktavianingsih@fmipa.unmul.ac.id

ABSTRACT

Citrus spp. is a locally grown fruit in Indonesia with high diversity and widespread distribution in the country. However, the diversity and similarities among *Citrus* spp. from North Penajam Paser Regency, East Kalimantan, have not been previously studied. Morphological characteristics were used in this study to provide data on the diversity and similarities of *Citrus* spp. from North Penajam Paser. The data provided served as a primary resource for further development and breeding. Additionally, observation, sampling, morphological data characterization, and scoring were carried out before the data was analyzed. This study identified 35 accessions of *Citrus* spp., including *Citrus* × *limon* (L.) Osbeck "Jeruk Lemon", *Citrus reticulata* Blanco "Jeruk Siam", *Citrus* × *aurantiifolia* (Christm.) Swingle "Jeruk Nipis", *Citrus* × *aurantium* L. "Jeruk Manis", *Citrus maxima* (Burm.) Merr. "Jeruk Bali", *Citrus* × *microcarpa* Bunge "Jeruk Kalamansi", and *Citrus* × *aurantium* L cv. Sweet Orange Group "Jeruk Sunkist", distributed across Penajam, Sepaku, Babulu, and Waru subdistricts. The dendrogram divided the species into two large clusters. Cluster A was based on characteristics, such as tree habitus, cordate leaf apex, entire leaf margin, pink mesocarp color, and the absence of seeds. Meanwhile, Cluster B was characterized by shrub habitus, acute and rounded leaf apex, crenate leaf margin, white to orange mesocarp color, and numerous seeds in the fruit. The diversity indicated the high potential value of *Citrus* spp. from North Penajam Paser for further development through a plant breeding program.

Keywords: *Citrus* spp, diversity, Kalimantan, morphological characters

INTRODUCTION

Indonesia is a mega biodiversity country, with an estimated 30,000 to 35,000 plant species dispersed across the landscapes (von Rintelen *et al.* 2017). The rich diversity is primarily attributed to several factors, including the equatorial proximity resulting in abundant rainfall, geological topographic variations, unique biogeographical conditions, diverse ecological conditions, and various climatic influences (Lohman *et al.* 2011). The distribution of numerous islands also leads to distinctive variations in the characteristics of species (Schouppe *et al.* 2017; Dart *et al.* 2012; Herlihy & Eckert 2005). This includes plants on Kalimantan Island, which is the third largest island in the world with enormous biodiversity.

Studying plant diversity and classification is critical in facilitating the development of economically valuable plant varieties or cultivars (Swarup *et al.* 2020). This field of study provides essential genetic information for the resources that form the foundation of plant breeding programs (Shen *et al.* 2018). Furthermore, examining plant diversity and similarity limits the analysis of morphological characteristics, including organ shape, size, color, and surface (Balduzzi *et al.* 2017).

Morphological characterization entails observing external features to assess and establish relationships among individuals, with each character assigned a value that can serve as a marker for assessing these relationships (Jensen 2009). Plants exhibit preferential traits that have evolved in response to various environmental conditions. Therefore, the study of morphological variation is essential as it shows the relationship with these environmental factors. Plants also possess an adaptive mechanism known as phenotypic plasticity, enhancing fitness and resilience in changing environmental conditions (Chevin *et al.* 2010; Valladares *et al.* 2014; Ayala *et al.* 2020).

Citrus spp. spread across Malay Archipelago, which includes Indonesia (Liu *et al.* 2012; Langgut 2017; Sofiyanti *et al.* 2022). These species are a valuable source of germplasm due to remarkable genetic diversity (Luro *et al.* 2011; Yu *et al.* 2018; Goh *et al.* 2022). *Citrus* spp. are also significant in local communities, serving as a vital source for various essential commodities. These include food, medicinal applications, and cosmetic formulations (Marti *et al.* 2009; Vijaylakshmi & Radha 2015; Samarina *et al.* 2021; Galovicová *et al.* 2022; Sapkota *et al.* 2022; Musara *et al.* 2023). Among the manifold applications, the fruits and leaves are frequently studied for potential cosmetics use, aromatic properties, and as indispensable ingredients in culinary and beverage preparations (Palazzolo *et al.* 2013; Khettal *et al.* 2016; Dosoky & Setzer 2018; Namani *et al.* 2018; Taufiq *et al.* 2019; Raghavan & Gurunathan 2021).

Citrus spp. has been considerably cultivated across various countries, with a remarkable presence in South East Asia. For instance, several studies found numerous distinct species in Indonesia, with many being hybrid products (Penjor *et al.* 2014; Susandarini *et al.* 2016; Yulian *et al.* 2021; Sofiyanti *et al.* 2022; Hardianto *et al.* 2023). "Jeruk Mandarin", which belongs to the *Citrus reticulata* Blanco, represents the most prevalent variety documented in Indonesia (Yulianti *et al.* 2020; Luro *et al.* 2023). "Jeruk Besar" refers to pomelos and is classified as *Citrus grandis* (L.) Osbeck (Ollitrault *et al.* 2020; Sofiyanti 2022), while "Jeruk Sitrun" is identified as *Citrus medica* (L.) Burm.f. (Benedetto *et al.* 2021).

Budiyati *et al.* (2016) carried out characterization to determine the diversity of *Citrus* spp. on Kalimantan Island, specifically in East Kalimantan, along with studies conducted in several other regions in the archipelago, such as Southeast Maluku, North Maluku, and East Java. Although the study in East Kalimantan was carried out generally in each region and was not specific for North Penajam Paser Regency, considerable variations were observed.

North Penajam Paser Regency was designated as the national capital of the Republic of Indonesia (Ibu Kota Nusantara) following Law Number 3 of 2022 concerning the National Capital (UU IKN) and the Regulatory Database (JDIH BPK RI) on 15 February 2022. This caused a massive change in the region, including the reduction and loss of plant germplasms due to the large number of new arrivals and simultaneous development that damaged the environment. The impact of these changes dramatically affected the use of plants. Therefore, this study aimed to provide phenetic diversity and similarity data for *Citrus* spp. from North Penajam Paser (IKN) for the benefit of local communities. The results are expected to serve as fundamental data for future studies.

MATERIALS AND METHODS

Observation and Sampling

This study was conducted from September 2021 to February 2022 in the North Penajam Paser Regency, East Kalimantan (Fig. 1). *Citrus* accessions were selected in this area and the observation site was selected based on potential location-based information provided by local communities. Sampling was carried out using the roaming method with a purposive sampling technique (Rugayah 2004). Leaves, flowering parts, fruits, and seeds that were observable were analyzed. All essential data were recorded using a camera to support the characterization. Analysis was carried out at the Laboratory of Plant Anatomy and Systematics, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan Province.

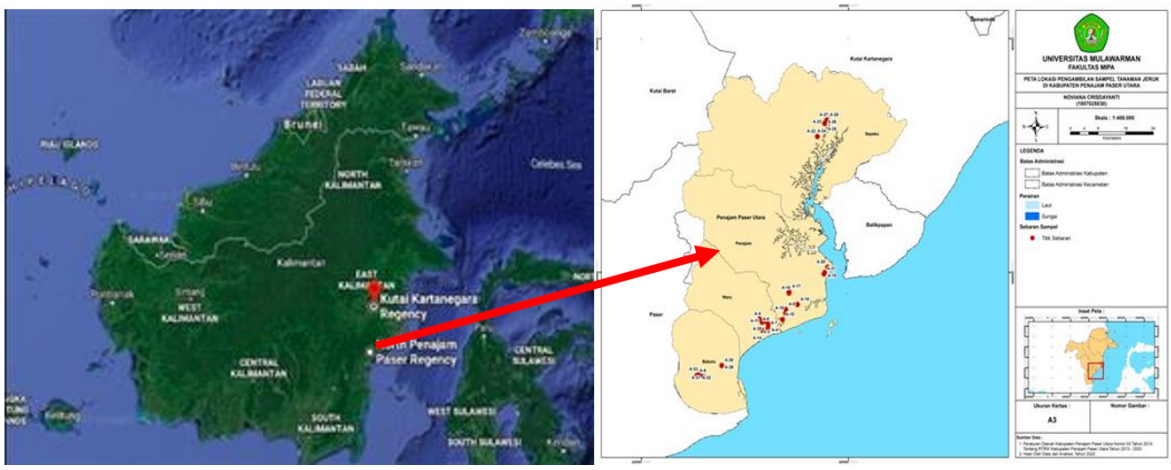

Figure 1 Location of collection of *Citrus* spp. accessions in North Penajam Paser Regency

Characterization and Scoring

Morphological characterization was conducted by analyzing the shape, length, texture, and color of the organs. A total of 50 critical characteristics were selected for characterization and converted into numerical values (Table 1) to assess diversity and similarity. The matrix score used in this analysis was based on Zufahmi and Nurlaila (2018) and IPGRI (1999). Each sample was assigned an accession number for identification purposes, and the identification process was carried out up to the species level.

Table 1 Conversion of morphological characters *Citrus* spp. for scoring

No	Characters	Scoring
1	Trunk ratio/rootstock diameter	0: 0 < 1 cm; 1: 1 = 1 cm; 2: > 1 cm
2	Scion trunk surface	0: Smooth; 1: Grooved
3	Tree shape	0: Ellipsoid; 1: Spheroid; 2: Obloid
4	Branch density	0: Sparse; 1: Medium; 2: Dense
5	Habitus	0: Shrub; 1: Tree
6	Stem color	0: Brown; 2: Green
7	Stem height	0: < 5 cm; 1: ≥ 5 cm
8	Stem shape	0: Perpendicular; 1: Perpendicular cylindrical
9	Stem surface	0: Thorny; 1: Flat
10	Spine length	0: ≤ 5 mm; 1: 6-15 mm; 2: 16-40 mm; 3: > 40 mm
11	Spine shape	0: Curved; 1: Straight
12	Shoot tip color	0: Green; 1: Purple; 2: Black; 3: Orange
13	Shoot tip surface	0: Glabrous; 1: Intermediate; 2: Pubescent
14	Leaf type	0: Sole; 1: Complex
15	Leaf apex	0: Acute; 1: Obtuse; 2: Rounded; 3: Cordate; 4: Sagittate
16	Leaf base	0: Acuminate; 1: Truncate
17	Leaf margin	0: Entire; 1: Crenate; 2: Sinuate
18	Leaf surface	0: Rough; 1: Smooth
19	Leaf shape	0: Ovate; 1: Elliptical
20	Leaf length	0: < 9 cm; 1: ≥ 9 cm
21	Leaf stalk	0: < 1.5 cm; 1: ≥ 1.5 cm

No	Characters	Scoring
22	Leaf width	0: < 5 cm; 1: ≥ 5 cm
23	Green Intensity	0: Light; 1: Medium; 2: Dark
24	Leaf lamina	0: Elliptic; 1: Ovate; 2: Obovate; 3: Lanceolate; 4: Orbicular; 5: Obcordate; 6: Oblong
25	Petiole wings	0: Absent; 1: Present
26	Petiole width	0: Narrow: 1: Medium; 2: Broad
27	Petiole shape	0: Obcordate; 1: Obdeltate; 2: Obovate; 3: Linear
28	Fruit shape	0: Spheroid; 1: Ellipsoid; 2: Pyriform 3: Oblique; 4: Obloid; 5: Asymmetric
29	Fruit base shape	0: Necked; 1: Convex; 2: Truncate; 3: Concave; 4: Concave collared; 5: Collared with neck
30	Fruit apex shape	0: Acute; 1: Rounded; 2: Truncate; 3: Mammiform
31	Fruit diameter	0: < 33 cm; 2: ≥ 33 cm
32	Fruit skin (epicarp)	0: Green/yellow; 1: Red; 2: Dark yellow; 3: Orange; 4: Dark yellow; 5: Dark green; 6: Light green
33	Fruit surface texture	0: Rough; 1: Smooth; 2: Papillate; 3: Pitted 4: Bumpy; 5: Grooved
34	Fruit peel thickness	0: Dense; 1: Light
35	Mesocarp color	0: Pink; 1: Yellow; 2: White; 3: Green; 4: Orange; 5: Light orange
36	Fruit taste	0: Sweet; 1: Sour
37	Vesicle length	0: Short; 1: Long
38	Nature of oil glands	0: Very weak; 1: Conspicuous; 2: Strongly conspicuous
39	Crown color	0: White; 1: Yellowish; 2: Violet
40	Stamen length	0: < 1 m; 1: ≥ 1 cm
41	Number of stamens	0: < 4 each petal; 1: 4 each petal; 2: > 4 each petal
42	Amount of pollen	0: Low; 1: Normal; 2: High
43	Flower stalk length	0: < 1 cm; 1: > 1 cm
44	Flower type	0: Hermaphrodite; 1: Male; 2: Female
45	Number of seeds	0: Low; 1: High
46	Seed shape	0: Fusiform; 1: Clavate; 2: Cuneiform; 3: Ovoid; 4: Semi deltoid; 5: Spheroid; 6: Semi-spheroid
47	Seed surface	0: Smooth; 1: Wrinkled; 2: Hairy
48	Seed color	0: White; 1: Beige; 2: Yellowish; 3: Green; 4: Brown
49	Cotyledon color	0: White; 1: Bright yellow; 2: Bright green; 3: White and green; 4: Green; 5: Dark green; 6: Violet
50	Presence/absence of Seeds	0: Absent; 1: Present

Source: IPGRI (1999); Zufahmi and Nurlaila (2018).

Data Analysis

Morphological characters were analyzed qualitatively and quantitatively. The qualitative analysis entailed using descriptive methods, where specimens were directly observed and matched with references (IPGRI 1999; Irsyam & Chikmawati 2015; Zufahmi & Nurlaila 2018), while quantitative analysis was carried out by measuring specific dimensions with a ruler, respectively. The process began with character selection as Operational Taxonomy Units (OTUs), with each chosen character assigned a standardized score (Table 1). All 50 selected characters were used for observation and converted into

standardized values. Subsequently, the data were entered into MVSP 3.1 (MultiVariate Statistical Package) software, which was used to generate the dendrogram and similarity index. The dendrogram was constructed using the Unweighted Pair Group with Arithmetic Mean (UPGMA) method with the Euclidean coefficient.

RESULTS AND DISCUSSIONS

Diversity

In this study, *Citrus* spp. was found in Penajam, Sepaku, Babulu, and Waru at various altitudes, ranging from 1 masl to 40 masl (meters above sea level). There were 35 identified accessions classified into seven species of *Citrus*, namely *Citrus* × *aurantium* L. "Jeruk Manis", *Citrus* × *aurantiifolia* (Christm.) Swingle "Jeruk Nipis", *Citrus* × *microcarpa* Bunge "Jeruk Kalamansi", *Citrus reticulata* Blanco "Jeruk Siam", *Citrus maxima* (Burm.) Merr. "Jeruk Bali", *Citrus* × *limon* (L.) Osbeck "Jeruk Lemon", and *Citrus* × *aurantium* L. cv. Sweet Orange Group (Table 2).

Morphological Characteristics

The characterization of 35 *Citrus* spp. accessions was conducted using 50 morphological characters based on the IPGRI Descriptor for *Citrus* characterization guidebook (1999), Zulfahmi (2018), and Irsyam and Chikmawati (2015). These characters revealed variations in leaves, fruits, and seeds. However, due to the unavailability of flowers in some accessions, the characterization primarily focused on fruit and leaf characters. This approach was based on a study conducted by Susandarini *et al.* (2013), which also emphasized the morphological character analysis of leaf and fruit characteristics for the same reason.

Leaf (Folium)

Morphological variations in leaves included variations in leaf shape and color. Leaf shapes varied from ovate, obovate, lanceolate, and orbicular, to elliptic (Fig. 2). Leaf colors ranged from light green to dark green. *Citrus* × *limon* (L.) Osbeck and *Citrus* × *microcarpa* Bunge both exhibited ovate leaf shapes. *Citrus* × *aurantiifolia* (Christm.) Swingle had elliptic leaves, while *Citrus* × *aurantium* L. and *Citrus reticulata* Blanco featured lanceolate leaves. *Citrus maxima* (Burm.) Merr. had orbicular leaves and *Citrus* × *aurantium* L. cv. Sweet Orange Group displayed obovate leaves.

Leaf color ranged from light to medium and dark. Specifically, *Citrus* × *limon* (L.) Osbeck leaves were green to dark green with a rough surface, acute leaf apex, and crenate margin. *Citrus* × *aurantiifolia* (Christm.) Swingle leaves were medium green with a rough surface, obtuse apex, acuminate base, and crenate margin. *Citrus* × *microcarpa* Bunge leaves were dark green with a smooth surface, obtuse apex, truncate base, and entire margin. *Citrus* × *aurantium* L. cv. Sweet Orange Group leaves were medium green with a smooth surface, acute apex, truncate base, and crenate margin. *Citrus* × *aurantium* leaves exhibited a medium to dark green color with a smooth surface, acute apex, acuminate base, and crenate margin. *Citrus reticulata* Blanco leaves were medium to dark green, with an acute apex, truncate base, smooth surface, and crenate margin. Lastly, *Citrus maxima* (Burm.) Merr. leaves were dark green with a cordate apex, truncate base, smooth surface, and entire margin.

A distinguishing feature of *Citrus maxima* (Burm.) Merr. from other *Citrus* species was the presence of petiole wings. *Citrus* × *limon* (L.) Osbeck and *Citrus reticulata* Blanco lacked wings on the leaf stalks, while some *Citrus* species had leaf stalks with wings and others did not. Figure 2 depicts the morphological characteristics of *Citrus* leaves. Budiarto *et al.* (2017) emphasized the presence of petiole wings as a significant morphological character that distinguished *Citrus* species.

The results showed variations in leaf lamina shapes, which differed from previous studies. Sofiyanti *et al.* (2022) reported that the leaf lamina shapes of *Citrus* spp. from Riau varied between elliptic, oblong, obovate, ovate, and oblanceolate shapes. The discrepancies in the identified accessions were primarily due to differences in the study populations.

Previous studies also identified a greater number of *Citrus* spp. compared to this study. Species, such as *Citrus* × *amblycarpa* (Hassk) Ochse "Jeruk Saring", *Citrus* × *aurantium* L. "Jeruk Hantu", *Citrus hystrix* DC. "Jeruk Purut", *Citrus hystrix* DC. "Jeruk Sundai", *Citrus longilimon* Tanaka, and *Citrus medica* L. "Jeruk Pagar", were not found in this study. This indicated that the variation in *Citrus* species from Riau was more significant than the species from North Penajam Paser, East Kalimantan.

Table 2 List of *Citrus* spp, accession found in North Penajam Paser, East Kalimantan

Accession number	Species name	Local name	Sampling location	Utility	Altitude (masl)
A-1	*Citrus* × *limon* (L.) Osbeck	Jeruk Lemon	Waru	Foodstuffs, beverages, traditional medical applications	11
A-2	*Citrus* × *aurantium* L. cv. Sweet Orange Group	Jeruk Sunkist	Waru	Foodstuffs and beverages	15
A-3	*Citrus* × *limon* (L.) Osbeck	Jeruk Lemon	Waru	Beverages	16
A-4	*Citrus* × *microcarpa* Bunge.	Jeruk Kalamansi	Waru	Foodstuffs and beverages	16
A-5	*Citrus reticulata* Blanco	Jeruk Siam	Waru	Foodstuffs and beverages	19
A-6	*Citrus* × *aurantiifolia* (Christm.) Swingle	Jeruk Nipis	Waru	Foodstuffs, seasoning ingredients	14
A-7	*Citrus* × *microcarpa* Bunge.	Jeruk Kalamansi	Waru	Foodstuffs, seasoning ingredients	5
A-8	*Citrus* × *aurantium* L. cv. Sweet Orange Group	Jeruk Sunkist	Babulu	Foodstuffs	40
A-9	*Citrus reticulata* Blanco	Jeruk Siam	Penajam	Foodstuff	9
A-10	*Citrus* × *aurantiifolia* (Christm.) Swingle	Jeruk Nipis	Penajam	Seasoning ingredients	1
A-11	*Citrus maxima* (Burm.) Merr.	Jeruk Bali	Waru	Foodstuffs	12
A-12	*Citrus* × *limon* (L.) Osbeck	Jeruk Lemon	Babulu	Beverages	38
A-13	*Citrus* × *aurantium* L.	Jeruk Manis	Waru	Foodstuffs and beverages	28
A-14	*Citrus* × *aurantium* L. cv. Sweet Orange Group	Jeruk Sunkist	Waru	Foodstuffs and beverages	28
A-15	*Citrus* × *limon* (L.) Osbeck	Jeruk Lemon	Penajam	Beverages	4
A-16	*Citrus* × *aurantium* L.	Jeruk Manis	Penajam	Foodstuffs	29
A-17	*Citrus* × *microcarpa* Bunge.	Jeruk Kalamansi	Penajam	Seasoning ingredients	32
A-18	*Citrus* × *aurantium* L. cv. Sweet Orange Group	Jeruk Sunkist	Penajam	Foodstuffs	14
A-19	*Citrus* × *aurantiifolia* (Christm.) Swingle	Jeruk Nipis	Penajam	Foodstuffs, seasoning ingredients	4
A-20	*Citrus* × *aurantium* L.	Jeruk Manis	Penajam	Foodstuffs	8
A-21	*Citrus* × *aurantiifolia* (Christm.) Swingle	Jeruk Nipis	Penajam	Foodstuffs, seasoning ingredients	10
A-22	*Citrus* × *aurantium* L.	Jeruk Manis	Sepaku	Foodstuffs	11
A-23	*Citrus* × *microcarpa* Bunge.	Jeruk Kalamansi	Sepaku	Foodstuffs, seasoning ingredients	16
A-24	*Citrus* × *limon* (L.) Osbeck	Jeruk Lemon	Sepaku	Beverages, traditional medical applications	15
A-25	*Citrus* × *aurantium* L. cv. Sweet Orange Group	Jeruk Sunkist	Sepaku	Foodstuffs	6
A-26	*Citrus* × *aurantium* L.	Jeruk Manis	Sepaku	Foodstuffs, beverages	24
A-27	*Citrus maxima* (Burm.) Merr.	Jeruk Bali	Sepaku	Foodstuffs	12
A-28	*Citrus* × *aurantiifolia* (Christm.) Swingle	Jeruk Nipis	Sepaku	Foodstuffs, seasoning ingredients	3
A-29	*Citrus* × *limon* (L.) Osbeck.	Jeruk Lemon	Babulu	Foodstuffs, beverages	7

Accession number	Species name	Local name	Sampling location	Utility	Altitude (masl)
A-30	*Citrus* × *aurantium* L.	Jeruk Manis	Babulu	Foodstuffs	8
A-31	*Citrus* × *microcarpa* Bunge	Jeruk Kalamansi	Babulu	Foodstuffs, seasoning ingredients	13
A-32	*Citrus* × *aurantium* L. cv. Sweet Orange Group	Jeruk Sunkist	Babulu	Foodstuffs	13
A-33	*Citrus* × *aurantium* L. cv. Sweet Orange Group	Jeruk Sunkist	Babulu	Foodstuffs	32
A-34	*Citrus maxima* (Burm.) Merr.	Jeruk Bali	Waru	Foodstuffs	13
A-35	*Citrus* × *aurantiifolia* (Christm.) Swingle	Jeruk Nipis	Waru	Foodstuffs, seasoning ingredients	14

According to Yulianti *et al.* (2020), there were 255 *Citrus* varieties, consisting of 43% mandarin-type, 4% tangerine-type, 0.7% sweet orange-type, 14.3% sour orange-type, 15% pummelo-type, and 23% rootstock, lime, and lemon. The large number of developed varieties makes the classification and identification process relatively complex. Furthermore, *Citrus* spp. can easily generate new hybrids through intraspecies or interspecies hybridization (Sofiyanti 2022). The species propagate via apomixis, allowing distribution over a wide range (Wang *et al*. 2017). Apomixis produces seeds that contain embryos from the somatic part of the nuclear cell (Kepiro & Roose 2010). A single seed is capable of developing from 2 to 30 embryos (Koltunow 1993; Wang *et al*. 2017). This ability is known as polyembryony, and breeders must be particularly aware due to the beneficial and detrimental effects (Wang *et al*. 2017).

Fruit (Fructus)

This study identified limited variations in fruit shape among *Citrus* spp. from North Penajam Paser. The observed fruit shapes ranged from spheroid to obloid, with the majority being obloid. *Citrus* × *limon* (L.) Osbeck exhibited a spheroidal shape with a convex base, acute apex, a diameter below 33 cm, a rough surface, and a dark green color.

Citrus × *aurantiifolia* (Christm.) Swingle had an ellipsoid shape with a convex base, rounded apex, a diameter below 33 cm, a smooth surface, and a dark green color. *Citrus* × *microcarpa* Bunge was characterized by an obloid shape with a convex base, rounded apex, a diameter below 33 cm, a green or yellow color, and a smooth surface. *Citrus* × *aurantium* L. cv. Sweet Orange Group had an obloid shape with a convex base, rounded apex, a diameter below 33 cm, a dark-green color, and a rough surface. *Citrus* × *aurantium* L. featured an obloid shape with a convex base, rounded apex, a diameter below 33 cm, a dark-green color, and a rough surface. *Citrus reticulata* Blanco bore fruits with an obloid shape, truncated base, rounded apex, a diameter below 33 cm, a green or yellow color, and a smooth surface. *Citrus maxima* (Burm.) Merr. fruits were obloid in shape, had a concave base, rounded apex, a diameter above 33 cm, a green or yellow color, and a rough surface.

This study showed that *Citrus* spp. of the same species generally exhibited consistent fruit shapes. This resullt contradicted the study conducted by Dorji and Yapwattanaphun (2011), which observed variations in fruit characteristics, even among the same species of *Citrus*. For instance, variations were found in fruit shape and skin surface texture for *Citrus reticulata* Blanco "Mandarin" across different sampling locations. Similar results were also reported in a study on *Citrus maxima* (Burm.) Merr. "Jeruk Bali" conducted by Susandarini *et al.* (2013), which successfully observed variations in fruit characteristics, including fruit shape, apex, base, peel texture, the presence of oil glands on the peel, mesocarp color, number and arrangement of fruit segments, fruit flesh color, texture, as well as the number of seeds. However, *Citrus maxima* (Burm.) Merr. exhibited different mesocarp colorations, including white and pink (Fig. 3).

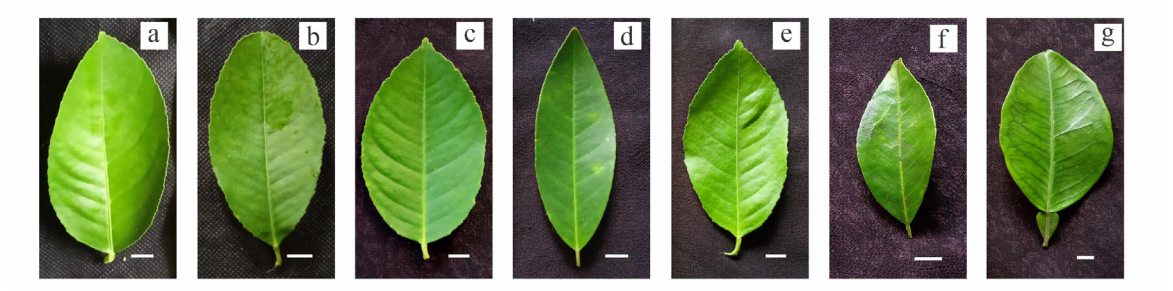

Figure 2 Leaf shapes

Notes: Without wing petioles, i.e., *Citrus* × *limon* (L.) Osbeck "Jeruk Lemon" (a) A-1; (b) A-3; (c) A-15; (d) A-24; (e) A-29 and *Citrus reticulata* Blanco "Jeruk Siam" (f) A-5.With wing petioles, i.e., *Citrus maxima* (Burm.) Merr. "Jeruk Bali" (g) A-34. Bar: 1 cm.

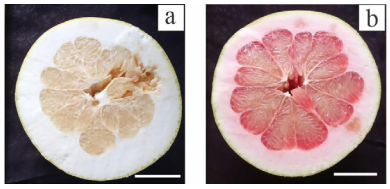

Figure 3 Variations in mesocarp color on the pomelo plant *Citrus maxima* (Burm.) Merr

Notes: (a) A-27 with white color; and (b) A-34 with pink color. Bar: 1 cm.

Mesocarp

The results showed variations in mesocarp color (Fig. 3), including pink, yellow, white, green, orange, and light orange. *Citrus* × *limon* (L.) Osbeck had a white mesocarp with a sour taste, prominent visible oil glands, and was seedless. *Citrus* × *aurantiifolia* (Christm.) Swingle exhibited a white mesocarp with a sour taste, prominent oil glands, and numerous seeds. *Citrus* × *microcarpa* Bunge exhibited a yellow mesocarp with a sour taste, faint oil glands, and was seedless. *Citrus* × *aurantium* L cv. Sweet Orange Group featured mesocarp in shades ranging from light orange to orange, a sweet taste, faint oil glands, and few seeds. *Citrus* × *aurantium* L. had orange mesocarp, a sweet taste, prominent oil glands, and few seeds. *Citrus reticulata* Blanco showed mesocarp in shades of yellow to orange, a sweet taste, faint oil glands, and numerous seeds. *Citrus maxima* (Burm.) Merr. had pink mesocarp with a sweet taste, faint oil glands, and was seedless.

The mesocarp is located between the epicarp and the endocarp (Sadka *et al.* 2019), constituting the pericarp. The outer part of the mesocarp may contain oil vesicles and carotenoid dyes (Szczykutowicz *et al.* 2020), and the concentration of these components may vary depending on *Citrus* species, environmental conditions, and other factors. Certain species showed high tolerance to specific environmental conditions. Furthermore, the interior of the mesocarp consists of spongy, white parenchyma tissue (Mabberley 2004).

Seed (Semen)

Variations in the shape of *Citrus* seeds include ovoid, semi-deltoid, club-shaped, semi-spheroid, spheroid, and irregular forms (Fig. 4). The seed shape does not necessarily correlate with the species of *Citrus*. However, cuneiform seeds are typically associated with the pomelo, *Citrus maxima* (Burm.) Merr (Accession 11 and Accession 27). The semi-spheroid seed shape is commonly found in the Siamese orange, *Citrus reticulata* Blanco "Jeruk Siam" (Accession 5). Irregular seed shapes are observed in *Citrus* × *limon* (L.) Osbeck (Accession 15 and Accession 24), and *Citrus* × *microcarpa* Bunge (Accession 23).

Cluster Analysis

Characterization of 35 *Citrus* spp. accessions using 50 morphological characters produced a dendrogram divided into two main clusters (Fig. 5). Cluster A contained three accessions, while Cluster B contains 32 accessions. The clustering was determined by the similarity of the characters, and the level of similarity determined the relationship of each accession. The similarity value increased with the number of identical characters. Cluster A had identical characteristics in tree habitus, cordate leaf apex, entire leaf margin, pink mesocarp color, and seed absence. Cluster B had similar characteristics in shrub habitus, acute and rounded leaf apex, crenate leaf margin, white to orange mesocarp color, and numerous seeds in the fruit.

Figure 4 The irregular shape of *Citrus* seeds

Notes: (a) A-15; (b) A-23; (c) A-24; (d) A-5; (e) A-6. Bar: 5 cm.

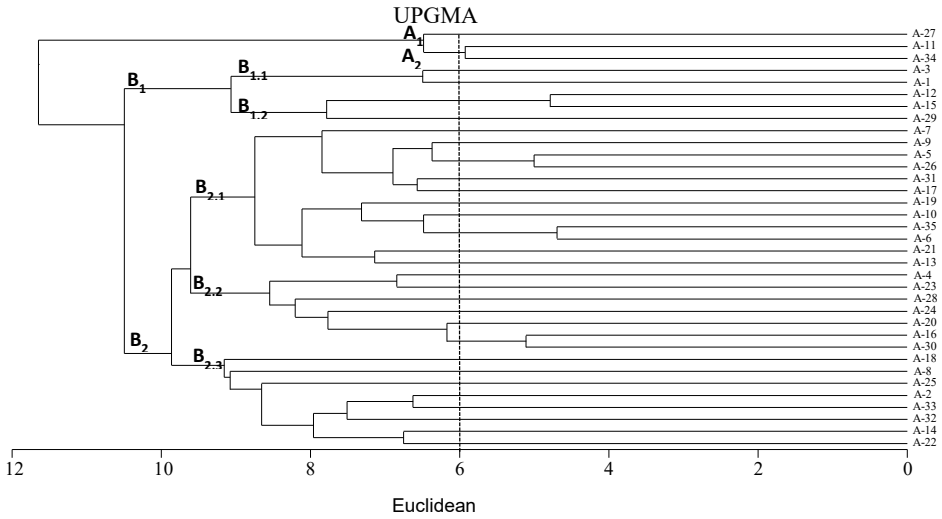

Figure 5 Dendrogram and clustering result of 35 *Citrus* spp. accessions from North Penajam Paser, East Kalimantan.

Each subcluster exhibited distinguishable characteristics that can be readily identified. Subcluster A was distinguished primarily by mesocarp color, and in this subcluster, there were variations in mesocarp color among the accessions. Specifically, subcluster A consisted of *Citrus maxima* (Burm.) Merr with varying mesocarp colors. Accession numbers A-11 and A-34 had pink mesocarp, while accession number A-27 had white mesocarp. Subcluster B comprised 32 accessions and was subdivided into two major groups based on several fruit-related characteristics, including fruit shape, fruit base shape, fruit apex shape, the nature of the fruit oil glands, and mesocarp and skin color. Subcluster B1 included *Citrus* × *limon* (L.) Osbeck which was further divided into two groups based on distinctive characteristics. These included the leaf apex color and the presence of seeds in the fruit. Subcluster B2 comprised *Citrus* × *microcarpa* Bunge, *Citrus* × *aurantiifolia* (Christm.) Swingle, *Citrus reticulata* Blanco, *Citrus* × *aurantium* L. "Jeruk Manis", and *Citrus* × *aurantium* L cv. Sweet Orange Group. The grouping in this cluster was primarily based on fruit shape, the nature of the oil glands, fruit apex shape, skin color, and mesocarp color. Generally, Subcluster B2 exhibited an obloid fruit shape, conspicuous oil glands, rounded fruit apex, and dense to thick mesocarp with varying colors.

PCA (Principal Component Analysis) was performed to support the clustering result (Fig. 6). This analysis showed the pattern of grouping accessions and determined the influential characters in the clustering result. The dendrogram results showed that the key characters for grouping *Citrus* spp. accessions are related to seed characteristics. These characteristics confined the presence or absence of seeds, seed shape, seed surface characteristics, and the number and texture of the seed surface. In addition, the position of each accession on PCA axis was consistent with the groupings observed in the dendrogram.

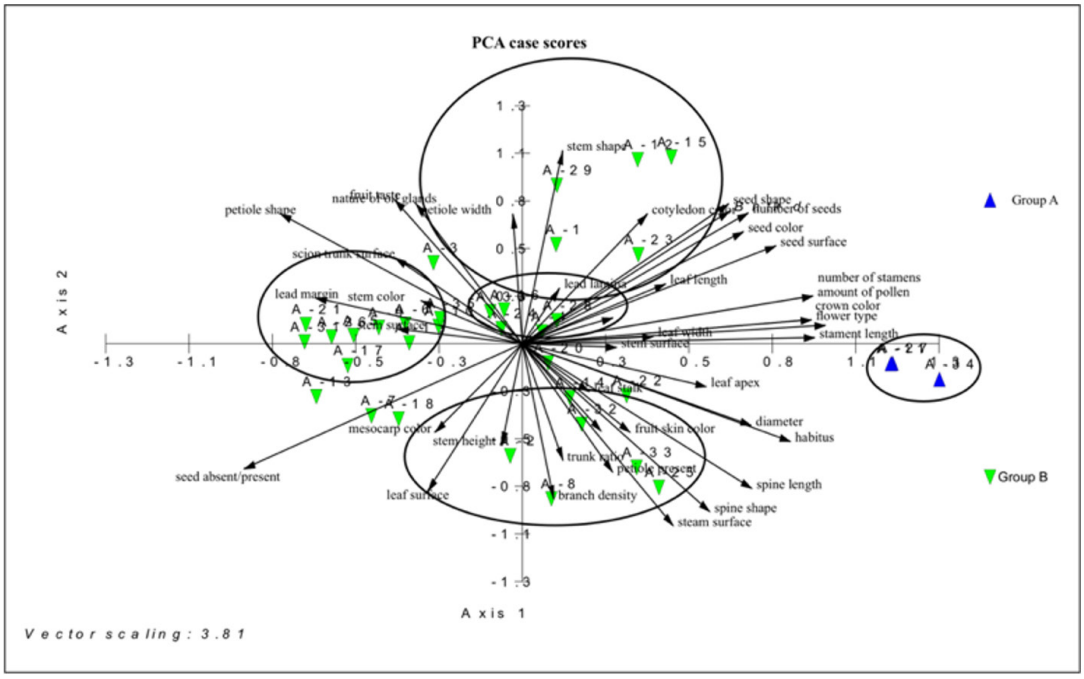

Figure 6 Principal Component Analysis of 50 characters and 35 *Citrus* spp. accessions from *North* Penajam Paser, East Kalimantan

CONCLUSION

Thirty five (35) accessions of *Citrus* spp. from North Penajam Paser Regency were successfully identified consisting of *Citrus* × *limon* (L.) Osbeck "Jeruk Lemon", *Citrus reticulata* Blanco "Jeruk Siam", *Citrus* × *aurantiifolia* (Christm.) Swingle "Jeruk Nipis", *Citrus* × *aurantium* L. "Jeruk Manis", *Citrus maxima* (Burm.) Merr "Jeruk Bali", *Citrus* × *microcarpa* Bunge "Jeruk Kalamansi", and *Citrus* × *aurantium* L cv. Sweet Orange Group "Jeruk Sunkist". *Citrus* spp. found in Penajam, Sepaku, Babulu, and Waru at varying altitudes of 1-40 masl. The dendrogram generated from 50 characters in this study was divided into two large clusters. Cluster A was grouped based on the tree habitus, cordate leaf apex, entire leaf margin, pink mesocarp color, and seed absence. Cluster B was grouped based on the shrub habitus, acute and rounded leaf apex, crenate leaf margin, white to orange mesocarp color, and numerous seeds in the fruit. The key characters for grouping *Citrus* spp. accessions were related to seed characteristics. *Citrus* spp. from North Penajam Paser had a great potential value for further development through plant breeding programs due to the high diversity. In addition, all components contributed to maintaining this diversity for the sustainability of biological resources that exist in North Penajam Paser.

REFERENCES

Ayala OA, Oyama K, Ríos-Muñoz CA, Rivas G., Barahona SR, Vega IL. 2020. Morphological variation of leaf traits in the *Ternstroemia lineata* species complex (Ericales: Penthaphylacaceae) in response to geographic and climatic variation. Peer J 8:e8307. DOI: 10.7717/peerj.8307

Balduzzi M, Binder BM, Bucksch A, Chang C, Hong L, Pascuzzi ASI, …, Sparks EE. 2017. Reshaping plant biology: Qualitative and quantitative descriptors for plant morphology. Front Plant Sci 8(76):117. DOI: 10.3389/fpls.2017.00117

Benedetto N, Carlucci V, Faraone I, Lela L, Poniticelli M, Russo D, …, Milella. 2023. An Insight into *Citrus medica* Linn.: A Systematic review on phytochemical profile and biological activities. Plants 12:2267. DOI: 10.3390/plants12122267

[BPS] Central Agency of Statistics (BPS). 2021. "Sepaku District in numbers 2021". BPS Catalog 1102001.6409040 [cited on 20 October 2023]. (in Indonesian).

Budiarto R, Poerwanto R, Santosa E, Efendi D. 2017. The potentials of Limau (*Citrus amblycarpa* Hassk. Ochse) as a functional food and ornamental mini tree based on metabolomic and morphological approaches. J Trop Crop Sci 4(2):49-57. DOI: 10.29244/jtcs.4.2.49-57

Budiyati E, Nirmala FD, Setiono. 2016. Utilization of Indonesian Orange Germplasm. J Agrovigor 9(1):9-66. DOI: 10.21107/agrovigor.v9i1.1526 (in Indonesian).

Chevin LM, Lande R, Mace GM. 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biol 8:e1000357. DOI: 10.1371/journal.pbio.1000357

Dart SR, Samis KE, Austen E, Eckert CG. 2012. Broad geographic covariation between floral traits and the mating system in *Camissoniopsis cheiranthifolia* (Onagraceae): Multiple stable mixed mating systems across the species' range? Ann Bot 109:599-611. DOI: 10.1093/aob/mcr266

Dorji K, Yapwattanaphun C. 2011. Morphological identification of mandarin (*Citrus reticulata* Blanco) in Bhutan. Kasetsart J Nat Sci 45:793-802.

Dosoky NS, Setzer WN. 2018. Biological activities and safety of *Citrus* spp. essential oils. Int J Mol. Sci 19:1966. DOI:10.3390/ijms19071966

Galovicova L, Borotova P, Vukovic NL, Vukic M, Kunova S, Hanus P, …, Kacaniova M. 2022. The potential use of *Citrus aurantifolia* L. essential oils for decay control, quality preservation of agricultural products, and anti-insect activity. Agron J 12:735. DOI: 10.3390/agronomy12030735

Goh RMV, Pua A, Luro F, Ee KH, Huang Y, Marchi E, …, Yu B. 2022. Distinguishing citrus varieties based on genetic and compositional analyses. PLoS ONE 17(4):e0267007. DOI: 10.1371/journal.pone.0267007

Hardianto, Devy NF, Sugiyanto A. 2023. Identification of morphological and physiochemical characteristics, flowering periods, and genetic relationship of *Citrus* species. IOP Conf. Series: Earth and Environmental Science 1172:012014. DOI: 10.1088/1755-1315/1172/1/012014

Herlihy CR, Eckert C.G. 2005. Evolution of self-fertilization at geographical range margins? A comparison of demographic, floral, and mating system variables in central vs. peripheral populations of Aquilegia canadensis (Ranunculaceae*).* Am J Bot 92:744-51. DOI: 10.3732/ajb.92.4.744

IPGRI. 1999. Descriptors for Citrus. Rome (IT): International Plant Genetic Resources Institute.

Irsyam ASD, Chikmawati T. 2015. Review of Genus *Citrus* (Rutaceae) in the Madura Region. Floribunda 5(3):82-91. DOI: 10.32556/floribunda.v5i3.2015.3 (in Indonesian).

Jensen RJ. 2009. Phenetics: Revolution, reform or natural consequence? Taxon 58:1-11.

Kepiro JL, Roose ML. 2010. AFLP markers closely linked to a major gene essential for nucellar embryony (apomixis) in *Citrus maxima* × *Poncirus trifoliata*. Tree Genet Genomes 6:1-11.

Khettal B, Kadri N, Tighilet K, Adjebli A, Dahmoune F, Benabdeslam FM. 2016. Phenolic compounds from Citrus leaves: antioxidant activity and enzymatic browning inhibition. J. Complement Integr Med 14(1). DOI: 10.1515/jcim-2016-0030

Koltunow AM. 1993. Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. Plant Cell 5:1425-37.

Langgut D. 2017. The citrus route revealed: From Southeast Asia into the Mediterranean. Hort Sci 52(6):814-22. DOI: 10.21273/hortsci11023-16

Liu YQ, Heying E, Tanumihardjo SA. 2012. History, global distribution, and nutritional importance of citrus fruits. Compr Rev Food Sci Food Saf 11:530-45. DOI: 10.1111/j.1541-4337.2012.00201.x

Lohman DJ, de Bruyn M, Page T, von Rintelen K, Hall R, Ng PKL, …, von Rintelen T. 2011. Biogeography of the Indo-Australian archipelago. Annu Rev Ecol Evol 42:205-26. DOI: 10.1146/annurev-ecolsys-102710-145001

Luro F, Gatto J, Costantino G, Pailly. 2011. Analysis of genetic diversity in *Citrus*. Plant Genet Resour 9(2):218-21. DOI: 10.1017/S1479262111000189

Luro F, Paoli M, Marchi E, Costantino G, Tomi F. 2023. Investigation of diversity by analyzing the polymorphism of ssr markers and the composition of leaf and fruit essential oils of 72 mandarins (*Citrus reticulata* Blanco). Horticulturae 9:577. DOI: 10.3390/horticulturae9050577

Mabberley DJ. 2004. *Citrus* (Rutaceae): A review of recent advances in etymology, systematics, and medical applications. Blumea: J Plant Taxon Plant Geogr 49:481-98. DOI: 10.3767/000651904X484432

Marti N, Mena P, Canovas JA, Micol V, Saura D. 2009. Vitamin C and the role of citrus juices as functional food. Nat Prod Commun 4(5):677-700.

Musara C, Aladejana EB, Mudyiwa SM. 2023. Review of the nutritional composition, medicinal, phytochemical and pharmacological properties of *Citrus reticulata* Blanco (Rutaceae). F1000Research 9:1387. DOI: 10.12688/f1000research.27208.1

Namani JA, Baqir E, Abri AA, Hubaishi TA, Husain A, Khan SA. 2018. Phytochemical screening, phenolic content and antioxidant activity of *Citrus aurantifolia* L. leaves grown in two regions of Oman. Iran. J Pharm Sci 14(1):27-34.

Ollitrault P, Curk F, Krueger R. 2020. *Citrus* Taxonomy. In Talon M, Caruco M, Gmitter FG (Editors). The Genus *Citrus*. Amsterdam (NL): Elsevier.

Palazzolo E, Laudicina VA, Germana MA. 2013. Current and potential use of *Citrus* essential oils. Curr Org Chem 17:3042-9. DOI: 10.2174/13852728113179990122

Penjor T, Mimura T, Matsumoto R, Yamamoto M, Nagano Y. 2014. Characterization of limes (*Citrus aurantifolia*) grown in Bhutan and Indonesia using high-throughput sequencing. Sci Rep 4:4853. DOI: 10.1038/srep04853

Raghavan S, Gurunathan J. 2021. *Citrus* species: A golden treasure box of metabolites that is beneficial against disorders. J Herb Med 28(2021):100438. DOI: 10.1016/j.hermed.2021.100438

Regulations Database (JDIH BPK RI). 2022. Law (UU) Number 3 of 2022 about the National Capital. https://peraturan.bpk.go.id/, accessed on 20 October 20 2023. (in Indonesian).

Rugayah, Retnowati A. Windadri FI, Hidayat A. 2004. Guidelines for collecting flora diversity data: Taxonomic data collection. Bogor (ID): Pusat Penelitian Biologi. (in Indonesian).

Sadka A, Shlizerman L, Kamara I, Blumwald E. 2019. Primary metabolism in Citrus fruit as affected by its unique structure. Front Plant Sci 10(1167):1-14. DOI: 10.3389/fpls.2019.01167

Samarina LS, Kulyan RV, Koninskaya NG, Gorshkov VM, Ryndin AV, Hanke MV, …, Reim S. 2021. Genetic diversity and phylogenetic relationships among citrus germplasm

in the Western Caucasus assessed with SSR and organelle DNA markers. Sci Hortic 288: 110355. DOI: 10.1016/j.scienta.2021.110355

Sapkota B, Devkota HP, Poudel P. 2022. *Citrus maxima* (Brum.) Merr. (Rutaceae): Bioactive chemical constituents and pharmacological activities. eCAM. 8741669. DOI: 10.1155/2022/8741669

Schouppe D, Brys R, Marin MV, Jacquemyn H. 2017. Geographic variation in floral traits and the capacity of autonomous selfing across allopatric and sympatric populations of two closely related Centaurium species. Sci Rep 7:46410. DOI: 10.1038/srep46410

Shen G, Girdthai T, Liu ZY, Fu YH, Meng QY, Liu FZ. 2018. Principal component and morphological diversity analysis of Job's-tears (*Coix lacryma-jobi* L.). Chil J Agric Res 79(1):131-43. DOI: 10.4067/S0718-58392019000100131

Sofiyanti N, Iriani D, Wahyuni PI, Idani N, Lestari P. 2022. Identification, morphology of *Citrus* L. (Aurantioideae-Rutaceae Juss.) and its traditional uses in Riau Province, Indonesia. Biodiversitas 23(2):1038-47. DOI: 10.13057/biodiv/d230247

Susandarini R, Subandiyah S, Rugayah, Daryono BS, Nugroho LH. 2013. Assessment of taxonomic affinity of Indonesian pummelo (*Citrus maxima* (Burm.) Merr.) based on morphological characters. Am J Agric Biol Sci 8(3):182-90. DOI: 10.3844/ajabssp.2013.182.190

Susandarini R, Rugayah, Nugroho LH, Subandiyah S. 2016. Chemotaxonomy of Indonesian Citrus maxima based on leaf essential oils. Am J Agric Biol Sci. 16 (1):26-33 DOI: 10.3844/ojbsci.2016.26.33

Swarup S, Cargill EJ, Crosby K, Flagel L, Kniskern J, Glenn CK. 2020. Genetic diversity is indispensable for plant breeding to improve crops. Crop Sci 61(2):839-52 DOI: 10.1002/csc2.20377

Szczykutowicz MK, Szopa A, Ekiert, H. 2020. *Citrus limon* (Lemon) Phenomenon: A review of the chemistry, pharmacological properties, applications in the modern pharmaceutical, food, and cosmetics industries, and biotechnological studies. Plants 9:119. DOI: 10.3390/plants9010119

Taufiq RM, Arry S, Hanif Z, Hardiyanto. 2019. Citrus in Indonesia: Production perspective for market development. Conference: International Symposia on Horticulture, "Emerging Challenges and Opportunities in Horticulture Supporting Development Goals"

Valladares F, Matesanz S, Guilhaumon F, Araújo MB, Balaguer L, Benito-Garzón M, …, Zavala MA. 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecol Lett 17:1351-64. DOI: 10.1111/ele.12348.

Vijaylakshmi P, Radha. 2015. An overview: *Citrus maxima.* J Phytopharm 4(5):263-7.

von Rintelen K, Arida E, Hauser C. 2017. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. Res Ideas Outcomes 3:e20860. DOI: 10.3897/rio.3.e20860

Wang X, Xu Y, Zhang S, Cao L, Huang Y, Cheng J, …, Qiang X. 2017. Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction. Nat Genet 49(5):765-73. DOI: 10.1038/ng.3839

Yu Y, Chen C, Huang M, Yu Q, Du D, Mattia MR, Gmitter FG. 2018. Genetic diversity and population structure analysis of *Citrus* germplasm with single nucleotide polymorphism markers. J Am Soc Hort Sci 143(6):399-408. DOI: 10.21273/JASHS04394-18

Yulian, Yunitasari, Romeida A, Marlin, Supanjani, Joko UK. 2021. Growth and development of shoot on lime (*Citrus hystrix*). Adv Biol Res 13:348-54. DOI: 10.2991/absr.k.210609.055

Yulianti F, Adiredjo AL, Soetopo L, Ashari S. 2020. Morphology and genetic characteristics of potential citrus rootstock in Indonesia. Biodiversitas 21(11):5514-20. DOI: 10.13057/biodiv/d211160

Zufahmi, Nurlaila. 2018. Relationships of Rutaceae family based on morphological characters in Bandar Baru District. *Prosiding Seminar Nasional Biotik*. ISBN: 978-602-604. (in Indonesian).