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LAMPIRAN



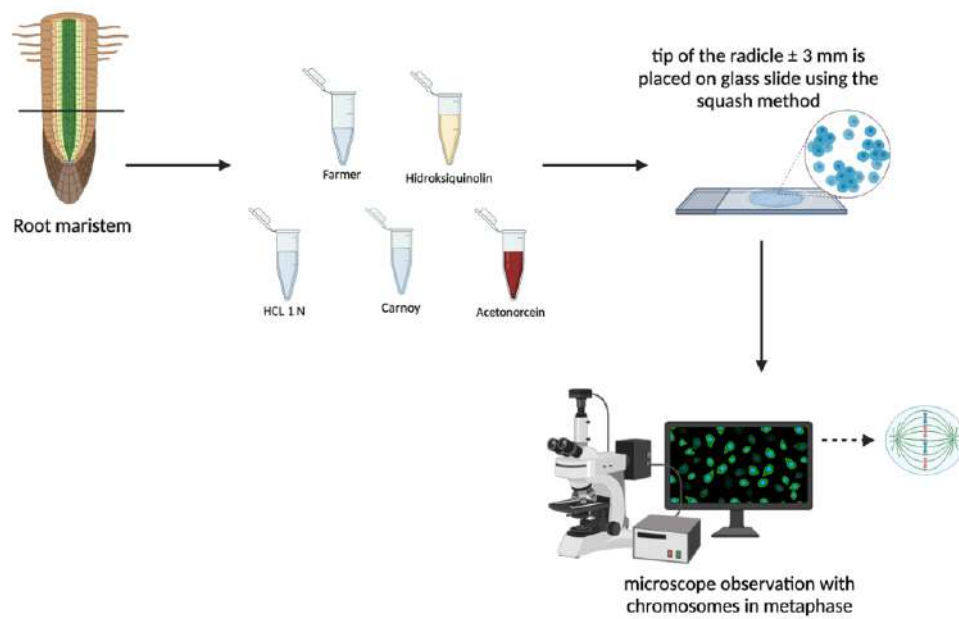
Gambar Lampiran 1. Foto lapangan yang menunjukkan karakteristik warna dan bentuk buah dari empat varietas *C. annum* var. *chinense* di Tana Toraja, Toraja Utara, Enrekang, dan Mamasa (A). Genotipe Limbong Sangpolo atau disebut Lokal di beberapa daerah (B). Genotipe Leatung 1 (C). Genotipe Leatung 2 (D). Genotipe Jumbo.



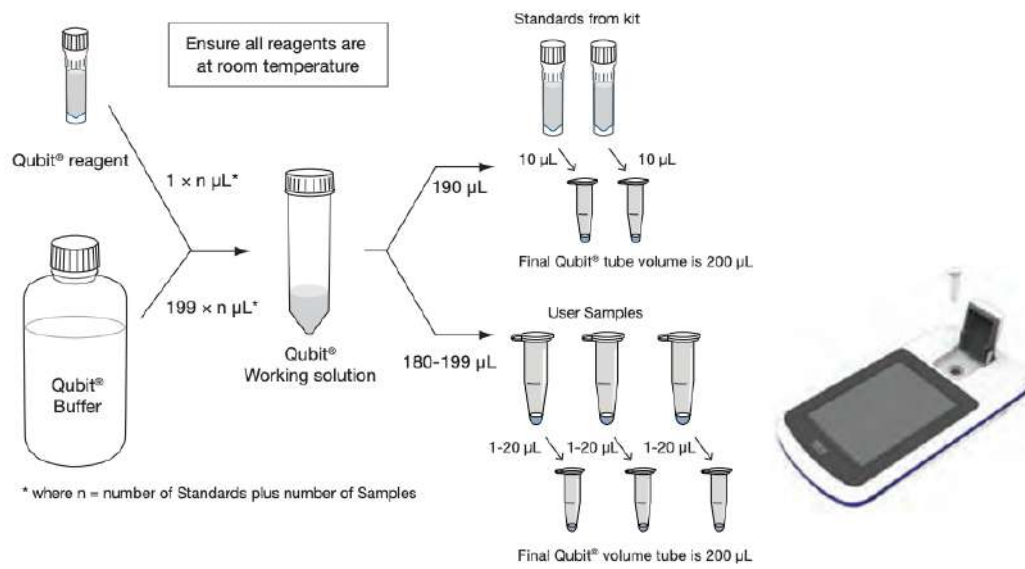
Gambar Lampiran 2. Biji semaian cabai katokkon disemai



Gambar Lampiran 3. Kondisi semaian cabai katokkon



Gambar Lampiran 4. Tahapan dalam analisis kromosom



Gambar Lampiran 5. Tahapan analisis kuantitas DNA sampel Katokkon

Tabel Lampiran 1. Sebaran pertanaman *C. annum var. chinense* cabai Katokkon di Kabupaten Toraja, Mamasa, dan Enrekang

Kabupaten	Kecamatan	Lembang Kelurahan	Kordinat		Ketinggian (MDPL)	Nama Varian
			E	S		
Toraja	Gandang Batu Silanan	Sillanan	119°51'0.648"	3° 11' 32.724"	1,363	Limbong Sangpolo (Local), Jumbo
Toraja	Kurra	Limbong Sangpolo	119°45'34.914"	2°57'29.274"	1,196	Limbong Sangpolo (local), Leatung I, Leatung II, Jumbo
Toraja	Makale	Lamunan, Rante	119°50'48.000"	3°6'35.000"	827	Limbong Sangpolo (local), Jumbo
Toraja	South Makale	Pasang, Tiromanda	119°48'48.204"	3°8'20.268"	1,230	Limbong Sangpolo (local), Jumbo, Leatung 1
Toraja	North Makale	Lemo	119°53'29.634"	3°3'18.036"	911	Jumbo
Toraja	Malimbong Balepe	Kole Sawangan	119°47'0.312"	3°4'44.904"	756	Limbong Sangpolo (Local), Jumbo
Toraja	Mengkendek	Buntu Datu	119°53'20.730"	3°15'1.410"	875	Jumbo
Toraja	Rantetayo	Tapparan, Madandan	119°48'28.503"	3°3'48.365"	837	Limbong Sangpolo (Local), Jumbo
Toraja	Rembon	Sarapeang, To Pao	119°46'45.696"	3°5'27.816"	818	Limbong Sangpolo (Local), Jumbo
Toraja	Saluputti	Pattan Ulus	119°45'27.492"	3°2'44.376"	849	Jumbo, Leatung I, Leatung II
Toraja	South Sangala	Tokesan	119°55'58.404"	3°7'15.384"	947	Limbong Sangpolo (Local), Jumbo
Toraja	Sangalla	Kaero	119°55'13.908"	3°5'11.400"	797	Limbong Sangpolo (Local)
Toraja	Buntao	Tongkonan Basse, Misa Ba'Bana, Rinding Kila'Balabatu	119°58'28.992"	3°2'23.784"	1,001	Limbong Sangpolo (Local), Leatung I, Leatung II, Jumbo
Toraja	Kapala Pitu	Kapala Pitu, Sikuku'	119°50'20.616"	2°56'11.676"	1,564	Limbong Sangpolo (Local), Leatung I, Leatung II, Jumbo
Toraja	Kesu	Tallu Lolo	119°53'11.874"	2°59'56.166"	798	Limbong Sangpolo (Local), Jumbo
Toraja	Nanggala	Nanggala Sangpiak Salu	119°59'34.884"	2°57'7.728"	876	Limbong Sangpolo (Local), Jumbo
Toraja	Rantebua	Buangin	119°58'58.800"	3°3'18.000"	714	Limbong Sangpolo (Local), Jumbo
Toraja	Rantepao	Limbong North	119°51'41.976"	2°57'10.446"	1,311	Leatung I
Toraja	Rindingalo	Pangala	119°48' 42.894"	2°54'24.552"	1,206	Limbong Sangpolo (Local), Leatung I
Toraja	Sanggalangi	Buntu La'Bo	119°56'12.588"	3°1'50.808"	807	Limbong Sangpolo (Local)
Toraja	Sesean	Deri	119°54'24.599"	2°54'52.301"	1,092	Limbong Sangpolo (Local), Jumbo
Toraja	Sopai	Salu	119°51'8.000"	2°58'18.000"	906	Limbong Sangpolo (Local), Jumbo
Toraja	Tallunglipu	Rante Paku	119°54'50.328"	2°58'10.776"	841	Limbong Sangpolo (Local)

Kabupaten	Kecamatan	Lembang Kelurahan	Kordinat		Ketinggian (MDPL)	Nama Varian
			E	S		
Mamasa	Sumarorong	Tadisi, Sumarorong, Rante Kamase, Banea	119°18'57.488"	3°8'57.346"	901	Limbong Sangpolo, Jumbo, Leatung 1
Mamasa	Mamasa	Rambu Saratu, Lambanan	119°23'38.103"	2°55'44.774"	1,162	Limbong Sangpolo
Enrekang	Curio	Buntu Barana, Pabaloran	119°53' 58.587"	3°17'18.058"	915	Limbong Sangpolo, Leatung 2,
Enrekang	Baroko	Tongko, North Benteng Alla	119°47'44.345"	3°16'52.656"	1,732	Jumbo, Limbong Sangpolo, Leatung 1, Leatung 2
Enrekang	Alla	Pana'	119°50'30.928"	3°16'26.347"	832	Jumbo, Leatung 2

Note: Geneotipe Limbong Sangpolo lebih dikenal sebagai genotype Lokal oleh petani.

Tabel Lampiran 2. Bahan-bahan mini kit Genaid Tanaman.

No.	Bahan	Volume
1	GP1 Buffer	2 mL
2	GPX1 Buffer	2 mL
3	GP2 Buffer	1 mL
4	GP3 Buffer	1,5 mL
5	W1 Buffer	2 mL
6	Wash Buffer (Penambahan ethanol)	4 mL
7	Elution Buffer	1 mL
8	Rnase A (10 mg/ml)	25 uL
9	Filter Columns	4
10	GD Columns	4
11	2 ml Colletion tubes	8

Tabel Lampiran 3. Larutan Reaksi PCR Polymerase Chain Reaction (PCR) untuk 1x reaksi.

No.	Larutan stok	Volume (µl)
1	Nanopure (ddH ₂ O)	3
2	Kappa	6,25
3	Primer	1,25
4	DNA	3
Volume total		13,5

Tabel Lampiran 4. Pembuatan larutan TAE stok Agarose.

No.	Larutan stok	Volume (mL)
1	Nanopure (ddH ₂ O)	490
2	TAE pekat	10
	Volume total	500

Tabel Lampiran 5. Pembuatan larutan Agarose berbasis 2%.

No.	Agarose 2%	Volume
1	Agarose	3,6 gram
2	TAE stok	180 mL
3	Gelred	1,6 μ l

Tabel Lampiran 6. Pembuatan larutan TAE Bak Elektrofotesis.

No.	Larutan	Volume
1	Nanopure (ddH ₂ O)	1.980 mL
2	TAE pekat	20 mL
	Volume total	2.000 mL

Tabel Lampiran 7. Pembuatan larutan TAE rendaman Agarose 2x pakai.

No.	Larutan	Volume
1	Nanopure (ddH ₂ O)	455 mL
2	TAE	5 mL
	Volume total	500 mL

Tabel Lampiran 8. Pembuatan Marker

Vol.	Larutan	(x)	Volume (μ l)
18	Nanopure (ddH ₂ O)	X2	36
4	Loadingdey	X2	8
2	Leader	X2	4
	Volume total		48

Tabel Lampiran 9. Pengaturan power supply elektroforesis

No.	Larutan	Volume
1	Tegangan	120 v
2	Curret	400 mA
3	Power	50 A
4	Waktu	80 menit

Tabel Lampiran 10. Elektroforesis uji kualitatif.

No.	Larutan	Volume (μ l)
1	DNA	2
2	Loadingdye	1
	Volume total	3

Tabel Lampiran 11. Kode DNA sampel cabai Katokkon

Kode DNA	Kode Lapangan	Kode DNA	Kode Lapangan
1	E1	14	M1
2	E2 (1)	15	M2
3	E2 (2)	16	M3
4	E2 (3)	17	M4
5	E3 (1)	18	E4 (1)
6	E3 (2)	19	M5
7	E3 (3)	20	M6
8	E4 (2)	21	M7
9	E5 (1)	22	M8
10	E5 (3)	23	T53
11	E6 (1)	24	T54
12	E6 (2)	25	T55 (1)
13	E6 (3)	26	T55 (2)

Keterangan: E (Enrekang), M (Mamasa), T (Toraja)

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1. Hafizhah Al-Amanah, Rinaldi Sjahril, Feranita Haring, dan Siti Halima Larekeng (2022). Molecular Diversity Analysis of Katokkon (*Capsicum annum* var. *chinense* based on Inter Simple Sequence

Repeat (ISSR) markers. *AGRIVITA*, Journal of Agricultural Science. Review

2. Hafizhah Al-Amanah, Rinaldi Sjahril, Feranita Haring, Muh Riadi, dan Siti Halima Larekeng (2022). Mapping distribution of *Capsicum annum* var. *chinense* in Tana Toraja and surrounding districts (Indonesia) based on fruit morphology. *Biodiversitas*, 23(2), 982–991. <https://doi.org/10.13057/biodiv/d230241>

E. Makalah pada Seminar/ Konferensi Ilmiah Nasional dan Internasional

- 1) Hafizhah Al-Amanah, Rinaldi Sjahril, Feranita Haring, dan Siti Halima Larekeng (2022). Primer Screening Based on ISSR and RAPD Markers for Amplification of Katokkon Pepper in Tana Toraja and Surrounding District, Indonesia. The 5th International Conference On Science Development in Science and Technology towards Better Quality of Life/ AIP Conference Proceedings. Accepted

Mapping distribution of *Capsicum annum* var. *chinense* in Tana Toraja and surrounding districts (Indonesia) based on fruit morphology

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Abstract. Al-Amanah H, Sjahri R, Haring F, Riadi M, Larekeng SH. 2022. Mapping distribution of *Capsicum annum* var. *chinense* in Tana Toraja and surrounding districts (Indonesia) based on fruit morphology. *Biodiversitas* 23: 982-991. Katokkon pepper (*Capsicum annum* var. *chinense*) is one of the many *Capsicum* genetic resources still cultivated in small farms and home gardens in Tana Toraja, North Toraja, Enrekang and Mamasa districts of Indonesia. This study was conducted to identify genetic diversity of *C. annum* var. *chinense* pepper based on fruit characteristics as morphological markers in the endemic area. The study location was determined by purposive sampling. Morphological characterization was carried out by observing quantitative and qualitative characters. Results showed that *C. annum* var. *chinense* was widely distributed in the districts of Tana Toraja 47% (14 sub-districts, 17 villages), North Toraja 34% (11 sub-districts, 14 villages), Enrekang 12% (3 sub-districts, 5 villages), and Mamasa 7% (2 sub-districts, 6 villages). Cluster analysis based on quantitative characters of fruit morphology from 51 accessions led to 5 dendrogram clusters with 90% similarity level. Principal Component Analysis (PCA) results had a cumulative diversity value of 75.913%. The color characteristic of fruit before ripening was light green, which turned red when mature. Cross-sections of the fruits were slightly corrugated. Fruits were pendant, and shapes were blocky. Fruit shape at pedicel attachment was truncated. Fruit shapes at the blossom end were blunt. In all the areas explored, only four common genotypes, i.e. Limbong Sangpolo or Local, Leatung 1, Leatung 2, and Jumbo were found.

Keywords: *Capsicum*, cluster analysis, conservation, diversity, fruit morphology

Abbreviations: BB: fruit weight; DB: fruit diameter; E: Enrekang; GPS: Global Positioning System; M: Mamasa; PB: fruit length; PCA: Principal component analysis; PT: stem length; T: Tana Toraja; TU: North Toraja; TB: flesh thickness; UPGMA: Unweight Pair Group Method with Arithmetic Mean Technique

INTRODUCTION

Pepper plants have a large diversity of species, as well they treasure various uses also. Although pepper is not the leading economic crop, several countries, including Indonesia, have recognized this plant as one of the most widely used plants by the local people. The utilization and cultivation of this plant are also diverse; hence this plant has significant economic value (Yamamoto et al. 2013).

Pepper is the most important horticultural commodity, and it is widely used as an ingredient in the kitchen and the food, beverage, pharmaceutical, and cosmetic industries (Sousa et al. 2015). Researchers have identified over 35 species of peppers in the world (Garcia et al. 2016); however, farmers have widely cultivated five species only, including *Capsicum annum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens*. These species are critical and research is needed to be focused on their breeding programs (Ibiza et al. 2012). In addition, testing seed quality and germination using an easy method are also necessary (Kusumawardana et al. 2018). Diversity studies

on pepper are reported to be helpful using fruit morphology (Albrecht et al. 2012).

Katokkon (*Capsicum annum* var. *chinense*) pepper is a species of chili pepper only found and cultivated in Tana Toraja District and its surrounding in the province of South Sulawesi, Indonesia. However, its commercial potential has not been widely exploited. *C. annum* var. *chinense* pepper is a commodity that the Toraja people are most interested in because of its distinctive aroma and a very spicy taste which comes from its capsaicin content (Marano et al. 2017). Capsaicin is found in the placenta, seeds and fruit flesh (Sahid et al. 2020), and the level of the pungency of pepper fruits is determined by the content of capsaicin (Simonovska et al. 2016). Due to the hot and spicy taste, *C. annum* var. *chinense* pepper is considered one of the favorite food ingredients in Tana Toraja, especially for spicy food enthusiasts. The extraordinary spicy taste is also what makes the price relatively high in the market. The community still cultivates these endemic plant genetic resources for consumption and commercial use. The *C. annum* var. *chinense* pepper has a unique shape similar to

the bell pepper type of paprika but is smaller (Marano et al. 2017). Peppers, however, also have ornamental potential due to differences in plant size, color, and fruit shape when grown in pots or in the garden (Padilha and Barbieri 2016). *C. annuum* var. *chinense* pepper has a high social value, and the Toraja people include this pepper in culinary practice for a certain rituals, ceremonies and festivals.

The origin of the *C. annuum* var. *chinense* pepper plant is still debatable. As far as our knowledge, the first seeds could have been brought by the Portuguese or the Dutch missionaries. However, they are now locally adapted. Their existence has begun to spread in other areas due to the migration of the Toraja ethnic communities to North Toraja, Enrekang, and Mamasa Districts. Pepper is also reported to achieve optimal spiciness in the mountains with an altitude of about 600-1,200 meters above sea level (Flowrenzhy and Harijati 2017).

Availability of genetic resources and appropriate breeding methods are important in assembling new high-yielding *C. annuum* var. *chinense* pepper varieties (Wijaya et al. 2020). Genetic diversity is essential to fulfilling the purpose of breeding crops since it is a level that refers to the total variation in the overall number of species (Peñuela et al. 2021). We can use high genetic diversity as a reference for selecting parents to assemble new varieties and enrich genetic diversity (Belay et al. 2019). Genetic diversity is the basis for increased germplasm availability and subsequent utilization during a breeding program (Larekeng et al. 2020). Plant breeding can improve the desired quality/traits of plants (Olatunji and Afolayan 2019).

Exploring local wisdom is known to provide in-depth bioresource-based traditional knowledge and the source of traditionally conserved genetic resources. The *C. annuum* var. *chinense* pepper plants are known to be conserved by Toraja ethnic communities in high mountainous areas. However, the lack of information about the diversity and taxonomic status of the *C. annuum* var. *chinense* pepper until now has made its conservation status and prospects unclear. Many reports are still not clear about the different naming of the *C. annuum* var. *chinense* pepper, making it seem contradictory because it is only based on morphological characters of *C. chinense* or *C. annuum* (Flowrenzhy and Harijati 2017; Tammu et al. 2021; Larekeng et al. 2019; Sjahril et al. 2020; Wätjen et al. 2021).

This report aims to identify and map the distribution of *C. annuum* var. *chinense* pepper genetic diversity based on specific morphological markers in Tana Toraja District and surroundings. The report is a part of research on the genetic diversity of *C. annuum* var. *chinense* pepper based on the molecular marker ISSR that aims to confirm the species name of the *C. annuum* var. *chinense* pepper growing in Tana Toraja District and the surroundings.

MATERIALS AND METHODS

Study area

This research was conducted using survey and observation methods in four districts as the center of *C.*

annuum var. *chinense* pepper cultivation, i.e., Tana Toraja District (3°5'23.49"S, 119°44'33.37"E), North Toraja District (2°52'39.78"S, 119°50'3.78"E), Enrekang District (3°28'33.31"S, 119°52'47.26"E), and Mamasa District (2°58'25.35"S, 119°21'59.51"E). The location was determined by purposive sampling based on information obtained from the agriculture office, and field officers from the Agriculture Extension, and local residents for *C. annuum* var. *chinense* local resources.

Sample collection

The sample was collected from 14 sub-districts of Tana Toraja, 11 sub-districts of North Toraja, 3 sub-districts of Enrekang, and 3 sub-districts of Mamasa. Each district was surveyed according to information on the existence of *C. annuum* var. *chinense* pepper farms or gardens. *C. annuum* var. *chinense* pepper fruit and plant samples were observed in situ. Fruit samples (10 fruits) per plant were taken directly from the farmer's land for morphological characterization and photograph. Seeds were counted for each fruit and placed in paper bags for storing during exploration.

Morphological characteristics analysis

The fruit characters observed were quantitative, i.e. fruit weight, fruit diameter, fruit length, stalk length, and fruit thickness. Whereas the qualitative character observed includes fruit color before ripening and at the mature stage, the character of the fruit position, calyx margin, fruit shape at the blossom end, fruit cross-section, and fruit shape at pedicel attachment. The characterization carried out was based on descriptors for *Capsicum*.

Data analysis

The findings of the location of *C. annuum* var. *chinense* pepper farms were marked using a GPS (Global Positioning System) device and converted into a map using the ArcGIS version 10.8 application. Data were analyzed using the Unweight Pair Group Method with Arithmetic Mean Technique (UPGMA) cluster and the Principal Component Analysis (PCA) method using the Multivariate Statistical Package program (MVSP) ver. 3.22.

RESULTS AND DISCUSSION

Mapping distribution of *Capsicum. annuum* var. *chinense* pepper

The locations of fruit samples taken from four districts of Tana Toraja, North Toraja, Enrekang, and Mamasa is shown in Figure 1, where we can see that the numbers of survey sites were dominantly in Tana Toraja and North Toraja. Geographically, the four districts are interrelated, where Tana Toraja and the North Toraja Districts are the centers of trade (Tammu et al. 2021). The same study on the *Capsicum annuum* in Tunisia showed that the high morphological diversity was due to the relationship between locations or accessions from adjacent areas and different areas. Intra-regional diversity can be a source of

inter-regional diversity useful for increasing pepper genetic information (Lahbib et al. 2012).

The point locations of the distribution map of *C. annuum* var. *chinense* pepper (Figure 1) are tabulated in Table 1 and their percent distribution across four districts are shown in Figure 2. Tana Toraja was found to have the most district and villages whose farmers used to grow *C. annuum* var. *chinense* (47%), followed by North Toraja District (34%), Enrekang District (12%), and Mamasa District (7%). As the North Toraja District was originally a single big district including Tana Toraja District until the expansion in the year 2008, it can be inferred that the center of *C. annuum* var. *chinense* pepper growing area is actually in Tana Toraja (3° 5'23.49"S, 119°44'33.37"E). Our interview with the farmers and agriculture extension officers has indicated that the *C. annuum* var. *chinense* pepper has traditionally been cultivated in Toraja for four generations. Yamamoto et al. (2014) postulated that some of the Capsicum species might have been introduced to Indonesia sometime in the late 1930s or The World War II. Lyrics of many Toraja folklore songs such as 'Dolong-dolong', which is also available in YouTube, tell the story about *C. annuum* var. *chinense* pepper (Iskandar 2018).

The *C. annuum* var. *chinense* pepper distribution found during our survey (Table 1) also had an interesting relationship with altitude. For instance, in Tana Toraja, they grow approximately at 797-1,363 m above sea level

(asl.), whereas in North Toraja it is approximately at 714-1,564 m ASL. Meanwhile, in Mamasa District it was only found in two locations in the range of 901-1,162 m asl. and in Enrekang at 832- 1,732 m asl. These data suggested that the mean range for optimal *C. annuum* var. *chinense* pepper growing altitude to be between 766-1,360 m asl. Interestingly, a location at Latimojong mountain (119° 47' 44.345"E, 3° 16' 52.656"S) with an altitude of 1,732 m asl, mean temperature 17°C (AccuWeather 2021) was situated far above the highest mean altitude calculated (E4S2).

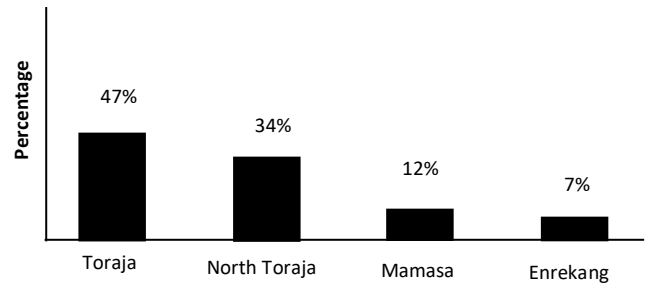


Figure 2. The percentage of distribution *C. annuum* var. *chinense* pepper found in four districts in Tana Toraja, North Toraja, Mamasa, and Enrekang of South Sulawesi Province, Indonesia

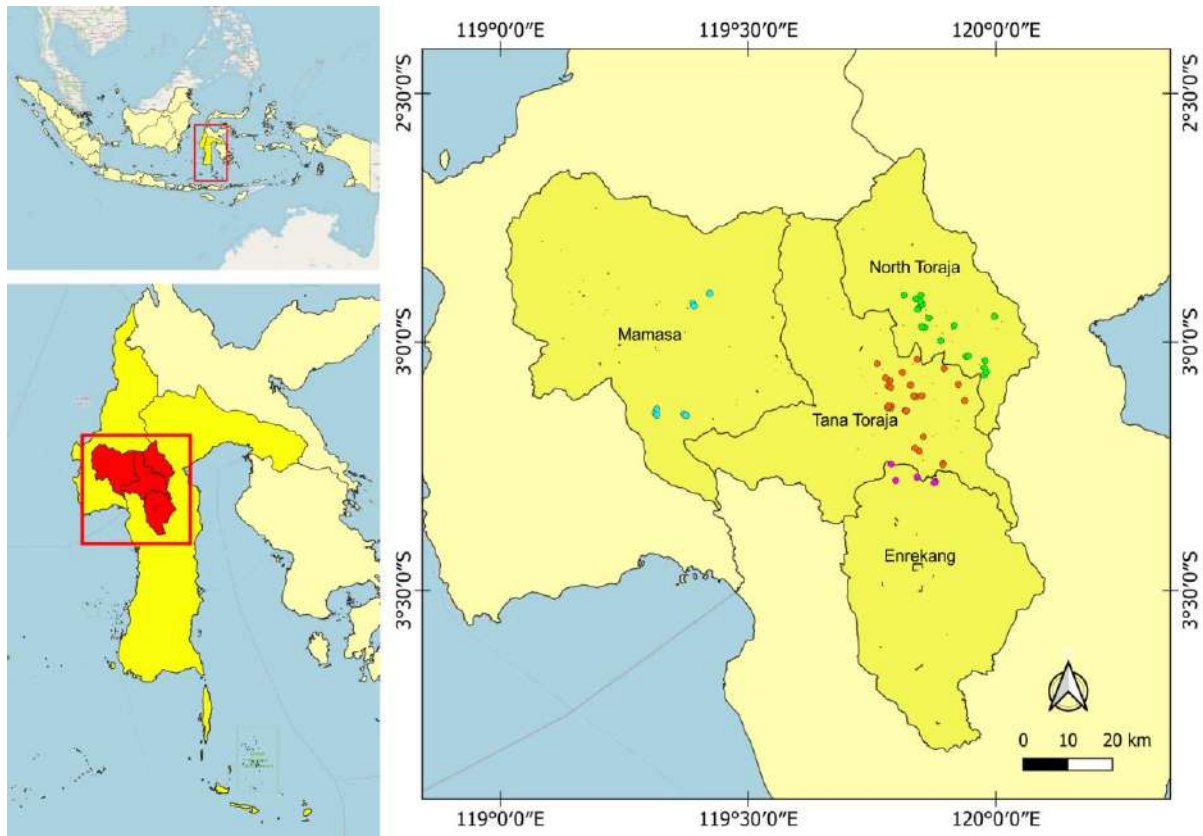


Figure 1. Distribution map of *C. annuum* var. *chinense* pepper in four districts of Tana Toraja, North Toraja, Mamasa, and Enrekang of South Sulawesi Province, Indonesia

Table 1. Distribution of *C. annum* var. *chinense* pepper in four districts in Tana Toraja, North Toraja, Mamasa, and Enrekang districts of South Sulawesi Province, Indonesia

Districts	Sub-districts	Village	Geographic coordinates		Elevation (m asl.)	Genotype
			E	S		
Tana Toraja	Gandang Batu	Sillanan	119°51'0.648"	3° 11' 32.724"	1,363	Limbong Sangpolo (Local), Jumbo
Tana Toraja	Kurra	Limbong Sangpolo	119°45'34.914"	2°57'29.274"	1,196	Limbong Sangpolo (local), Leatung I, Leatung II, Jumbo
Tana Toraja	Makale	Lamunan, Rante	119°50'48.000"	3°6'35.000"	827	Limbong Sangpolo (local), Jumbo
Tana Toraja	South Makale	Pasang, Tiromanda	119°48'48.204"	3°8'20.268"	1,230	Limbong Sangpolo (local), Jumbo, Leatung I
Tana Toraja	North Makale	Lemo	119°53'29.634"	3°3'18.036"	911	Jumbo
Tana Toraja	Malimbong Balepe	Kole Sawangan	119°47'0.312"	3°44'4.904"	756	Limbong Sangpolo (Local), Jumbo
Tana Toraja	Mengkendek	Buntu Datu	119°53'20.730"	3°15'1.410"	875	Jumbo
Tana Toraja	Rantetayo	Tapparan, Madandan	119°48'28.503"	3°3'48.365"	837	Limbong Sangpolo (Local), Jumbo
Tana Toraja	Rembon	Sarapeang, To Pao	119°46'45.696"	3°5'27.816"	818	Limbong Sangpolo (Local), Jumbo
Tana Toraja	Saluputti	Pattan Ulus	119°45'27.492"	3°2'44.376"	849	Jumbo, Leatung I, Leatung II
Tana Toraja	South Sangala	Tokesan	119°55'58.404"	3°7'15.384"	947	Limbong Sangpolo (Local), Jumbo
Tana Toraja	Sangalla	Kaero	119°55'13.908"	3°5'11.400"	797	Limbong Sangpolo (Local)
North Toraja	Buntao	Tongkonan Basse, Misa Ba'Bana, Rinding, Kila'Balabatu	119°58'28.992"	3°2'23.784"	1,001	Limbong Sangpolo (Local), Leatung I, Leatung II, Jumbo
North Toraja	Kapala Pitu	Kapala Pitu, Sikuku'	119°50'20.616"	2°56'11.676"	1,564	Limbong Sangpolo (Local), Leatung I, Leatung II, Jumbo
North Toraja	Kesu	Tallu Lolo	119°53'11.874"	2°59'56.166"	798	Limbong Sangpolo (Local), Jumbo
North Toraja	Nanggala	Nanggala Sangpiak Salu	119°59'34.884"	2°57'7.728"	876	Limbong Sangpolo (Local), Jumbo
North Toraja	Rantebua	Buagin	119°58'58.800"	3°3'18.000"	714	Limbong Sangpolo (Local), Jumbo
North Toraja	Rantepao	Limbong	119°51'41.976"	2°57'10.446"	1,311	Leatung I
North Toraja	Rindingalo	North Pangala	119°48' 42.894"	2°54'24.552"	1,206	Limbong Sangpolo (Local), Leatung I
North Toraja	Sanggalangi	Buntu La'Bo	119°56'12.588"	3°1'50.808"	807	Limbong Sangpolo (Local)
North Toraja	Sesean	Deri	119°54'24.599"	2°54'52.301"	1,092	Limbong Sangpolo (Local), Jumbo
North Toraja	Sopai	Salu	119°51'8.000"	2°58'18.000"	906	Limbong Sangpolo (Local), Jumbo
North Toraja	Tallunglipu	Rante Paku	119°54'50.328"	2°58'10.776"	841	Limbong Sangpolo (Local)
Mamasa	Sumarorong	Tadisi, Sumarorong, Rante Kamase, Banea	119°18'57.488"	3°8'57.346"	901	Limbong Sangpolo, Jumbo, Leatung I
Mamasa	Mamasa	Rambu Saratu, Lambanan	119°23'38.103"	2°55'44.774"	1,162	Limbong Sangpolo
Enrekang	Curio	Buntu Barana, Pabaloran	119°53' 58.587"	3°17'18.058"	915	Limbong Sangpolo, Leatung 2,
Enrekang	Baroko	Tongko, North Benteng Alla	119°47'44.345"	3°16'52.656"	1,732	Jumbo, Limbong Sangpolo, Leatung I, Leatung 2
Enrekang	Alla	Pana'	119°50'30.928"	3°16'26.347"	832	Jumbo, Leatung 2

Note: Limbong Sangpolo genotype is more commonly recognized by farmers as a Local genotype

Morphological quantitative characteristics analysis

Cluster analysis is used to group data obtained in the field into several classes with grouping criteria based on the size of the dissimilarity. We can measure the dissimilarity between objects using the Euclidean distance measure. The closer or smaller the Euclidean distance between genotypes, the more similar the genotypes are (Habtie et al. 2017).

The cluster analysis divided the 51 accessions data into 5 clusters (Figure 3; Table 2). Cluster I comprise one accession (E4S2). Cluster II comprises of 12 accessions (T33, T12S2, E2S1, E3S2, E2S3, TU29S1, TU15, E4S1, E2S2, T32, T23 and E5S1). Cluster III comprises 2 accessions (T55S1 and T40). Cluster IV consists of 30 accessions (T34, T24, T2, M5, M6, T52, M2, TU29S2, M7, E6S1, T1, M1, T27, T50, T11, T7S2, TU30S2, T53, E6S2, T25, T7S1, M8, E3S1, TU26, E1, TU14, T55S2, T54, T46

and T22). Cluster V consists of 6 accessions (TU30S3, T12S1, TU30S1, T6, M4 and M3). Based on the fruit's morphological characters and the planting location, it showed 5 clusters with a 90% similarity level.

The differences in genotype groups can determine the similarities and kinship between them. Genotypes that are in the same group have similarities and are closely related. Genotypes that are in different groups have featured quite unique to the group members. Expanding genetic diversity crosses between closely related genotypes will produce higher diversity than closely associated ones (Zhang et al. 2016).

Taxonomists begin the classification process by identifying species morphologically. Morphological character identification is a straightforward and rapid procedure applied directly to plant populations (Susilowati et al. 2020). Larekeng et al. (2019), in their research with *Tectona grandis* has observed tree organs such as leaves,

stems, flowers, fruits, canopies, resin, and bark for comparing the trees. Results of morphological identification also reflect the species' relatedness (Herison et al. 2018). Based on their research on *C. annum* var. *chinense* pepper, Sjahril et al. (2020) also showed a variety of morphological characteristics. The shape of the fruit tip and the shape of the fruit base show grooves dominate them. The second cluster shares qualitative morphological characteristics with the first cluster, including a dark green leaf color and petals with jagged edges.

Principal component analysis (PCA) is a method used to simplify data by transforming the data linearly to determine the characteristics that affect plant genetic diversity (Deka et al. 2016). The data from the analysis shows that there are characters that make positive and negative contributions to the quadrant grouping. This shows that characters with positive values contribute significantly to the diversity of the same group and the reverse for characters with negative values (Singh et al. 2020). They reported the same phenomenon using PCA on pepper plants, which showed that the morphological

characters could be the source of genetic diversity in the *Capsicum annum* group (Devi et al. 2017).

Based on the bi-plot analysis (Figure 4), the dots indicate accession, while the vector shows the variable morphological component of the fruit. PC1 and PC2 explained a cumulative diversity value of 75.913%. We divide the functional diversity group into four quadrants to show the tendency of accession to a variable. Quadrant I on the bi-plot comprised of 16 accessions (E1, E6S2, M1, M3, M4, M6, T11, T12S1, T46, T52, T53, T54S1, T55S2, TU14, TU29S2, TU30S1) with variable fruit weight (BB), fruit diameter (DB), and flesh thickness (TB). Quadrant II consists of 11 accessions (E3S1, E6S1, M2, M7, M8, T6, T7, T22, T55S1, TU26, TU30S3) with variable stem length (PT) and fruit length (PB). Quadrant III of 13 accessions (E2S1, E2S2, E3S2, E4S2, M5, T2, T24, T27, T34, T50, T40, TU15, TU30S2) and quadrant IV consists of 11 accessions (E2, E5S1, E4S1, T1, T7, T12S2, T23, T25, T32, T33, TU29S1) The third and fourth quadrant did not have a specific character to characterized the accession.

Table 2. Groups of 51 accessions according to cluster analysis from five agronomics traits

Cluster	Accession
I	E4S2
II	T33, T12S2, E2S1, E3S2, E2S3, TU29S1, TU15, E4S1, E2S2, T32, E5S1, and T23
III	T55S1 and T40
IV	T34, T24, T2, M5, M6, T52, M2, TU29S2, M7, E6S1, T1, M1, T27, T50, T11, T7S2, TU30S2, T53, E6S2, T25, T7S1, M8, E3S1, TU26, E1, TU14, T55S2, T54, T46, and T22
V	TU30S3, T12S1, TU30S1, T6, M4, and M3

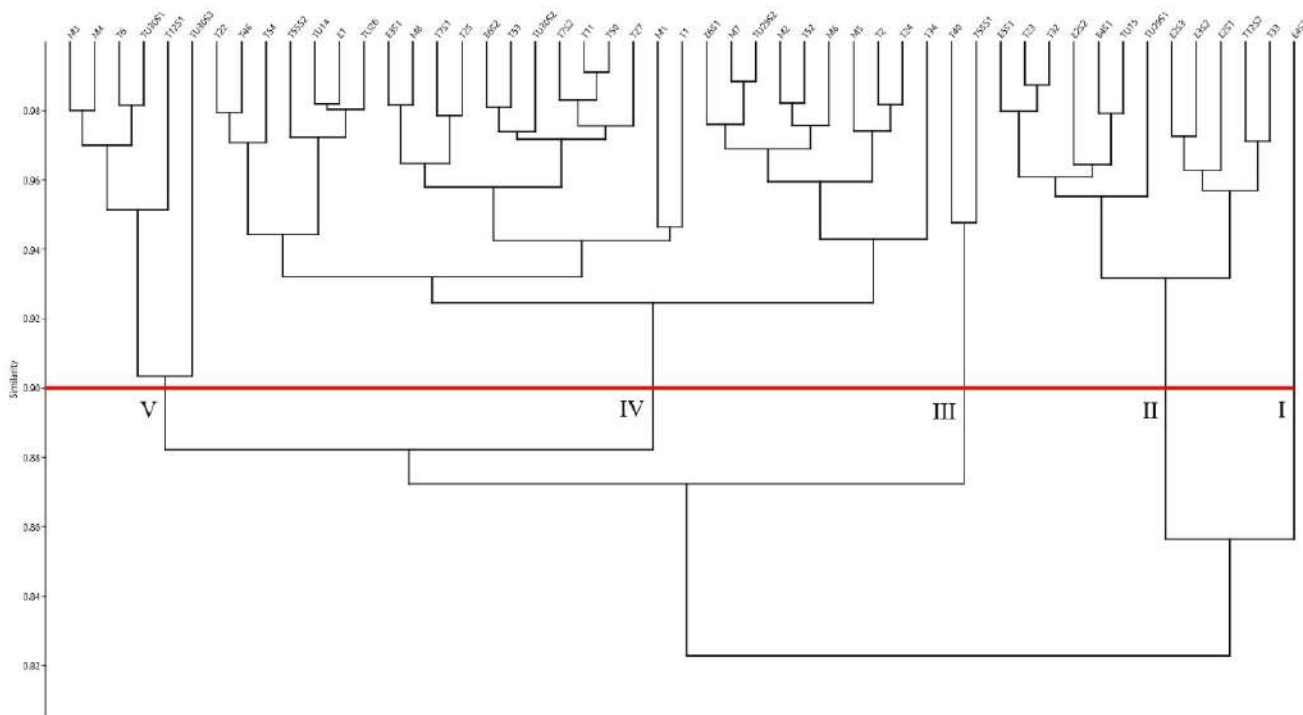


Figure 3. Cluster analysis showing the relationship among 51 accessions of *C. annum* var. *chinense* pepper based on five agronomic traits (fruit weight, fruit diameter, fruit length, stalk length, and fruit thickness)

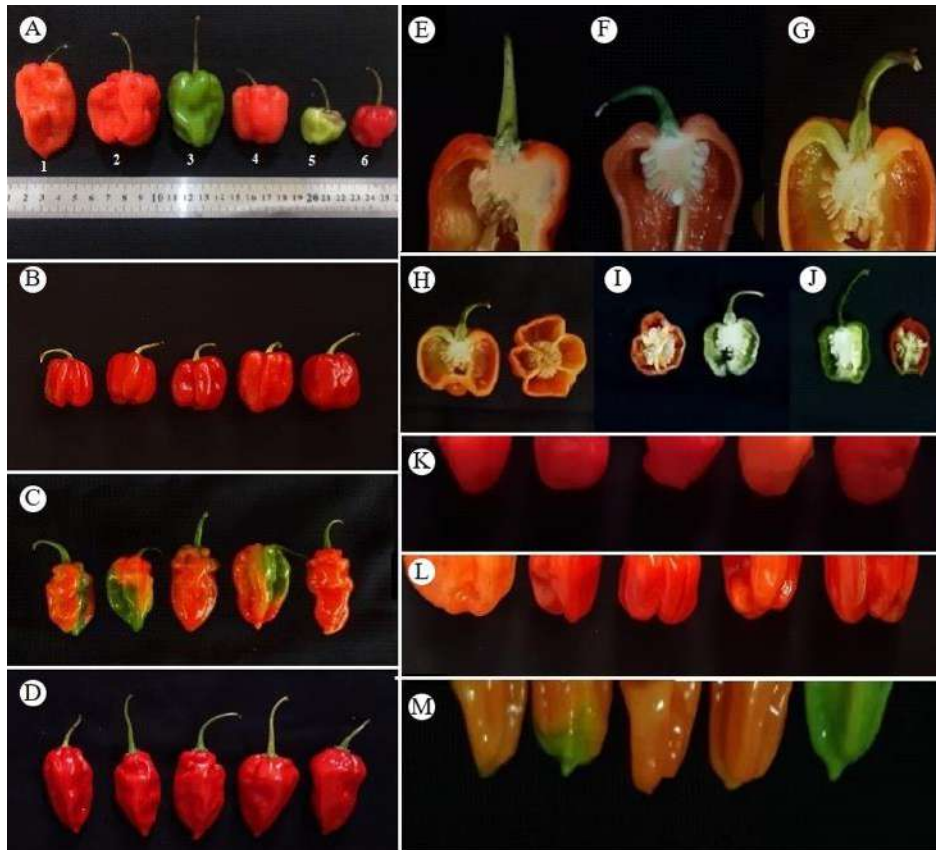


Figure 5. Qualitative character of *C. annuum* var. *chinense* pepper fruit. A. Light green, patches of purple, dark green fruit color before ripening and red fruit color at the mature stage, B. Blocky fruit shape, C. campanulate, D. Triangular, E. Corrugated cross-sections of fruit (F. Intermediate; G. Slightly corrugated; H. Truncate fruit shape at pedicel attachment; I. Cordate; J. Lobate; K. Blunt fruit shape at the blossom end; L. Sunken; M. Sunken and pointed. Pictures are not scaled except picture A.



Figure 6. Field photographs showing characteristics of fruit color and shape of four varieties of *C. annuum* var. *chinense* pepper grown in Tana Toraja, North Toraja, Enrekang, and Mamasa of South Sulawesi Province, Indonesia. A. Genotype Limbong Sangpolo or called Local in some areas, B. Genotype Leatung 1, C. Genotype Leatung 2, D. Genotype Jumbo.

Table 3. Grouping of the qualitative character of *C. annum* var. *chinense* pepper fruits 51 accessions found in Tana Toraja, North Toraja, Enrekang, and Mamasa Districts of South Sulawesi Province, Indonesia

Qualitative character	Accession
Fruit color before ripening is light green	T2, T7S1, T6, T12S1, T22, T24, T25, T34, T46, T40, T52, T54, T55S1, M6, M8, E1, E2S2, E3S1, TU26, TU29S2, TU30S3
Fruit color before ripening has patches of purple.	T1, T7S2, T11, T12S2, T23, T27, T32, T33, T50, T53, M1, M2, M3, M4, M5, M7, E2, E4 S1, E5S1, E6S1, TU14, TU15, TU29S1, TU5S2, TU30S1
Fruit color before ripening is dark green	T55S1, E2S3, E3S2, E4S2, E6S2
Fruit color at the mature stage is red	All accession
Fruit cross-section is corrugated	T2, T6, T12S1, T27, T46, T54, T55S1, E1, E2S2, E3S1, E3S2, M1, M2, M8, TU14, TU29S1, TU29S2, TU30S1, TU30S2, TU30S3
Fruit cross-section is slightly corrugated	M5
Fruit cross-section is intermediate	T1, T7S1, T72, T11, T12S2, T22, T23, T24, T25, T32, T33, T34, T40, T50, T52, T53, T55S2, E2S1, E2S3, E4S1, E5, E6S1, E6S2, M3, M4, M6, M7, TU15, TU26
Fruit shape is blocky	T1, T2, T7S1, T7S2, T6, T12S2, T12S1, T22, T23, T25, T27, T23, T33, T46, T40, T50, T52, T53, T54, T55S1, M1, M2, M3, M4, M7, M8, E1, E2S1, E2S3, E3S1, E3S2, E4S1, E4S2, E5S1, E6S1, E6S2, TU14, TU15, TU26, TU29S1, TU30S1, TU30S2, TU30S3
Fruit shape is campanulate	T24, T55S2, M5, M6, E2S2, TU29S2
Fruit shape is triangular	T11, T34
Fruit shape at pedicel attachment is truncate	E2S1, E4S1, E5S1, E6S1
Fruit shape at pedicel attachment is cordate,	T2, T7S1, T7S2, T6, T12S2, T25, T27, T32, T34, T46, T40, T50, T52, E1, E2S2, E3S1, E6S2, M5, M6, TU14, TU15, TU29S1, TU29S2
Fruit shape at pedicel attachment is lobate	T1, T11, T12S1, T22, T23, T24, T33, T53, T54, T55S1, T55S2, E2S3, E3S2, E4S2, M1, M2, M3, M4, M7, M8, TU26, TU30S1, TU30S2, TU30S3
Fruit shape at blossom end is blunt	T1, T2, T7S1, T7S2, T6, T11, T22, T24, T33, T34, T40, T55S2, M1, M2, M3, M4, M7, M8, TU29S1, TU29S2
Fruit shape at blossom end is sunken	T12S2, T12S1, T23, T25, T27, T32, T46, T50, T52, T53, T54, T55S1, E1, E2S1, E3S1, E4S1, E5, E6S1, E6S2, M5, M6, TU14, TU15, TU26, TU30S1, TU30S2, TU30S3
Fruit shape at blossom end is sunken and pointed	E2S2, E2S3, E3S2, E4S2

The qualitative fruit characters observed above in Tana Toraja, North Toraja, Enrekang, and Mamasa Districts were also observed in young fruit. The morphological characteristic of fruit before ripening was light green, which turned red when mature. The fruit cross-section was slightly corrugated. The fruit was a pendant, and shapes were blocky. Fruit shape at pedicel attachment was truncated. Fruit shapes at the blossom end were blunt. There was no difference in fruit shape and color between different altitudes. However, there seemed to be a slight variance between the 51 accessions found, but it was not area or location-specific (Table 1; Figure 6). In almost all the regions surveyed, there were only four variants of genotypes that were mentioned by the respondents and the survey guides during our interviews. The genotype variants names were Limbong Sangpolo (some people name it “Local”), Leatung 1, Leatung 2, and Jumbo (Figure 6). This variance may be genetic because they can grow in all altitudes and locations without changing the shape and color of fruits. Qualitative characters are characters that have little or no effect on the environment and are characters that are controlled by simple genes (Wartono et al. 2019). To fortify the findings, further molecular studies may be required.

In this study, we concluded that *C. annum* var. *chinense* pepper plants were widely distributed in Tana Toraja District (47%), followed by North Toraja District (34%),

Enrekang District (12%), and in Mamasa District (7%). However, since the sites of the *C. annum* var. *chinense* pepper farms’ GPS coordinates were found close to each other at the border between Tana Toraja and North Toraja Districts and considering our interviews and literature, we propose that the center of growing and production of *C. annum* var. *chinense* pepper has been initiated in Tana Toraja.

Interestingly, in all the areas explored, we found only four common genotypes, i.e., Limbong Sangpolo or Local, Leatung 1, Leatung 2 and Jumbo. Further studies in this regard could be targeted with a molecular approach to analyze differences among those four genotypes of *C. annum* var. *chinense* pepper.

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ICo5 2022

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For the manuscript entitled

“Primer Screening Based on ISSR and RAPD Markers for Amplification of Katokkon Pepper in Tana Toraja and Surrounding District, Indonesia”

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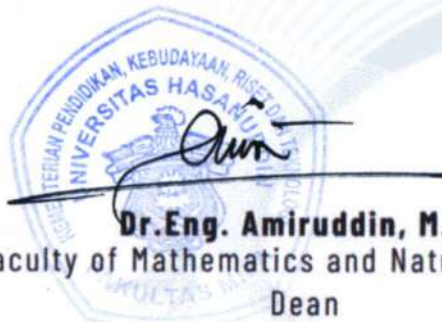
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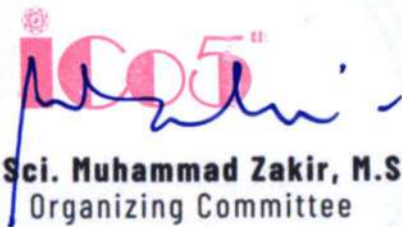
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Makassar, 18 September 2022

Dear : Hafizhah Al- Amanah, Rrinaldi Sjahril, Feranita Haring and Sitti Halimah Larekeng

This letter is to confirm that your paper entitled:

**Primer Screening Based on ISSR and RAPD Markers for Amplification of Katokkon
Pepper in Tana Toraja and Surrounding District, Indonesia**

IS ACCEPTED

By the committee of The 5thInternational Conference on Science 2022 (ICoS 2022) and ready
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Best regard,
On behalf of the Organizing Committee
The 5th ICoS 2022

Dr. Sci. Muhammad Zakir, M.Si
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