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Comparison of morphological characters, flowering phenology and essential oils of two accessions of *Cananga* (*Cananga odorata* var. *fruticosa*)

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Abstract. *Na'imah MNR, Kurniawati A, Kisantini. 2024. Comparison of morphological characters, flowering phenology and essential oils of two accessions of* Cananga *(*Cananga odorata *var.* fruticosa*). Biodiversitas 25: 801-810.* The *Cananga* flowers are well-known in various regions of Indonesia as a raw material for essential oils and flower decorations. This study aims to compare the characteristics of two varieties of *Cananga* originating from Kediri and Cipanas. Both types of *Cananga* were cultivated in the Institut Pertanian Bogor (IPB) Cikabayan experimental field. Observation was conducted on leaf and flower characteristics, flowering phenology, and essential oil content. The research shows that the Kediri accession exhibited brownish-red leaf petioles, brownish-red branches, and dark brown stems. In contrast, the Cipanas accession had green leaf stalks, green branches, and light brown stems. The flowering phenology of the Kediri accession spanned from the emergence of flower buds to the End stage, lasting 35 days, while the Cipanas accession's flowering period extended for 25 days. The Kediri accession's flowers have more petals and are longer and heavier than the Cipanas accession. The highest essential oil content of the Cipanas accession occurred at the 50% full flowering stage, reaching 0.92%, and gradually decreased as the flowering progressed. In comparison, the Kediri accession oil content is the highest at the end of the flowering stage, with a peak of 0.61%. The highest compound in the Kediri accession is all-trans-Farnesol acetate 14,24%, β-Copaene 12.23%, and Caryophyllene 15.92%. Meanwhile, in the Cipanas accession, the highest compound content was linalool 18.74% and caryophyllene 16.00%.

Keywords: Essential oil, heat unit, petals number, pigment, trichome

INTRODUCTION

According to the Ministry of Trade of the Republic of Indonesia (2022), essential oils are a potential commodity in realizing Indonesia's non-oil and gas exports. The essential oils provided a growth trend of 10% in Indonesia's potential non-oil and gas export commodities from 2017 to 2021. During this period, a total of 25 provinces exported essential oils with a total of 102 countries of export destination. Indonesia produces 40 of the 80 types of essential oils marketed worldwide. As many as kinds of essential oils have entered the world market, namely patchouli, vetiver, *Cananga*, cajuput, lemongrass, clove, sandalwood, nutmeg, cinnamon, cubeb, and pepper (Ministry of Trade of the Republic of Indonesia 2011).

Cananga odorata (Lam.) Hook. f. & Thomson belongs to the Annonaceae family (Saedi 2006). Some *Cananga* species worldwide include *C. odorata*, *Cananga latifolia* Finet & Gagnep., *Cananga scortechinii* King, and *Cananga brandisiana* (Pierre) I.M.Turner (Pujiarti et al. 2015). Geographically, the distribution of *C. odorata* covers Indonesia, Malaysia, southern Myanmar, the Philippines, and northern Australia (Parrotta 2018). *Cananga* that grows in Indonesia is *C. odorata* forma *macrophylla*, which is distributed on Java Island in several areas, now referred to as just *Cananga* (Hobir et al. 1990). In cananga cultivation development in Indonesia, farmers have cultivated types of *Cananga* other than *Cananga* forma *macrophylla*, namely *C. odorata* var. *fruticose* (Ramadhani and Salamah 2021)

The *Cananga* flower has a significant meaning for the Indonesian people. Besides being a raw material for essential oils, *Cananga* flowers have many benefits, both in fresh flowers and flower extracts. Fresh *Cananga* flowers can also processed into hair oil by heating *Cananga* flowers with coconut oil to produce *Cananga*-scented hair oil. On the island of Bali, fresh *Cananga* flowers are used to perfume hair, clothes, and bedding (Hatta 1993). The distillation of *Cananga* flowers produces essential oils, which are used as raw materials for aromatherapy (Ismail et al. 2020), antibacterial (Tan et al. 2015), antifungal and antioxidant (Upadhyay et al. 2021). *Cananga* oil can be used as aromatherapy to reduce anxiety disorders (Binoriang and Pramesti 2021).

Although the use of flowers has been developed for a long time and has become an industrial raw material, basic knowledge about flower characteristics, flowering phenology, and the potential of the oil produced must be discovered. Flowering phenology is essential in the plant life cycle, which is the initial plant reproduction (Trimanto et al. 2020). In the growth stage of *Cananga* flowers, Qin et al.

(2014) stated that there are seven stages of flowering development in *Cananga*, namely budding, petal appearance, initial flowering, full flowering, final flowering, wilted flower, and dry flower stages. The compounds released by *Cananga* flowers largely depend on the stage of flower development. In addition, in the inflorescence stage, there is an initial process for a plant to reproduce, which begins with the appearance of flower buds and ends with fruit ripening (Agustin et al. 2021). In addition, because *Cananga* flowers have economic value as raw materials for essential oils, the flowering phenology associated with the accumulation of essential oils is crucial to be studied.

The development of *Cananga* flowers can categorized into several stages, from buds to mature flowers. The stamens and pistils grow during the bud stage and mature following the petal emergence phase. (Ramadhani and Salamah 2021). Flower harvest criteria greatly determine the essential oil content of an oil producer. In addition, the flower development stage is also related to the fragrance of *Cananga* flower levels, the size of the flowers, and their aesthetic value as the flowers are used fresh. The variety of *Cananga* in Indonesia, such as in the central areas of *Cananga* production in East Java and other areas, is still not widely known. The characteristics of *Cananga* plant diversity and studies of flowering phenology associated with essential oil content are rare. Therefore, studying the flowering phenology associated with the accumulation of essential oils is crucial. This information helps determine the criteria for harvesting the best flowers for essential oil raw materials or fresh flowers.

MATERIALS AND METHODS

Study area

This research was conducted from September 2022 to October 2023 at the Cikabayan Experimental Field, Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor, with an altitude of 240 masl, coordinates 6°33'01" S and 106°42'51" E. Flower sampling starts from December 2022 to April 2023 (rainy season), essential oil distillation and GCMS analysis is carried out from January to October 2023. Daily temperature ranges from 21-34°C. The soil type is latosol, and the pH is 4.44.

Procedures

Experimental design

This research used a two-factor split-plot experimental design, with the main plot representing *Cananga* accessions and the subplots representing floral growth stadia. The *Cananga* accession combines the Kediri and Cipanas accessions; the Cipanas accession is collected from a region with more rainfall than the Kediri. Moreover, the flower growth stage consists of the initial flowering stage, the 25% full flowering stage, the 50% full flowering stage, the 75% full flowering stage, and the End flowering stage. The phase determination (flower growth phase) is based on Qin et al. (2014), states that there are seven flowering phases which are then simplified to five levels. The experiment was replicated five times so that there were 50 experimental units. Each experimental unit contained five plants, so there were 250 plants. The seedlings obtained are developed from vegetative propagation by grafting, and planting materials are planted using a spacing of 1×1 m. At the same time, watering is done after transplanting if there is no rain for two weeks. Mostly, no irrigation is needed, and the plants get water from rainwater. Initial fertilization is carried out before transplanting using manure (1 kg/planting hole), and NPK (16-16-16) fertilization at a dose of 10g/plant is carried out every two months.

Observations and measurements included morphological characters, flowering phenology, flower production, and essential oil content. Leaf morphology was observed on leaf color, and leaf length was measured from the tip of the leaf to the base of the leaf. Leaf width was measured at the most comprehensive distance between the two edges of the leaf. In addition, the ratio of length/width of the leaf was observed by comparing the results of the measurement of the length and width of the leaf with the criteria of the width being more prominent than twice the length, the leaves being rounded, the color of the stalk and the color of the central leaf veins. Stem morphology on the color of young and old stems was observed by looking at the differences in stem color of the two accessions. Flower morphology was observed on the petals' length by measuring the petals' length from the petals' base to the petals' tip. The number of petals was observed by counting the number of petals contained in one flower, the weight of the flowers was weighted on each flower in each developmental stage, and the color of the flowers was observed by comparing the color of each flowering stage referring to the Munsell Plant Tissue Color Book. Observation of plant morphology using three replications, each replication using five plants. Flower morphology observation using five replications with five samples per replication. Observation of leaf anatomy using three replications with two leaf samples per replication.

The observation material used for stomata and trichome leaves is completely opened and located on primary branches. Collecting leaf stomata, leaf trichomes, and flowers is done by attaching an adhesive tool using clear tape to the leaf's surface and rubbing it gently until the adhesive sticks perfectly. Next, apply nail coloring thinly and evenly to the dry nail polish leaves' bottom surface, remove them using insulation, and then stick them on the glass preparation. Observation of stomata and trichomes using a microscope with a magnification of 40× with a field of view of 2.8 units in diameter and a field of view of 0.5 mm^2. Analysis of leaf pigments in the form of chlorophyll, anthocyanin, and carotenoid used the Sims and Gamon method (2002). Organoleptic testing was carried out by testing 10 respondents to determine the flower aroma score. The flower aroma score is (1) not fragrant, (2) slightly fragrant, (3) fragrant, (4) very fragrant.

Observations of flowering phenology were carried out by calculating the time required from one stage of the flowering stage to the next. Flowering phenology observations were carried out by observing 5 replications

of each treatment, with 5 sample plants in each replication. Meanwhile, the volume of extracted essential oils is measured, and the essential oil content is calculated at each flowering stage. Extraction of essential oils was carried out by the boiled distillation method with distillation equipment, which was carried out by lowering the flowers in a kettle with water until all the flowers were submerged entirely and boiled for 6 hours to produce essential oils (Guenther 1987). The formula for obtaining essential oil content is (oil weight/sample weight) × 100%.

Observation of essential oil profiles using Shimadzu brand GC-MS. The sample was injected into the mass spectroscopy instrument using an autosampler of 200 µL. The results obtained from the GC-MS instrument are GC spectra, namely the retention time; mass spectra can be obtained for the relative molecular mass of each compound and abundance of the compound produced from the sample.

Data analysis

Observations on the morphological characters will describe the characteristics of each accession of *Cananga*. The quantitative character data of the vegetative stage were compared with the t-test at a 5% level; the quantitative data of the reproductive stage were tested with the F-test and further tested with the Honest Significant Difference (HSD) at a 5% level if significantly different were observed.

RESULTS AND DISCUSSION

Morphological character of *Cananga*

The morphology of the two *Cananga* accessions observed showed differences in the characters on the leaves and stems, which included leaf length, leaf width, petiole color, leaf's primary vein color, and branch color (Table 1). The general differences between the two *Cananga* plant accessions can be seen in Figure 1. The Kediri accession has greater leaf length and width than the Cipanas accession. *Cananga* leaves include single leaves with pinnate veins and a leaf length ratio of twice the leaf width, which is oval. The color of the two accessions' petioles is different; the petiole of the Kediri accession is brownish red. In contrast, the color of the petiole of the Cipanas accession is green (Figure 2). The Kediri accession has green main leaf veins from the tip of the leaf vein to the middle, and from the middle of the leaf vein to the petiole, it is brownish red. The Cipanas accession has a green primary vein color from the leaf vein's tip to the leaf's base.

The color of the branches between the two accessions also shows differences. The Kediri accession is brownish red, while the Cipanas accession is green (Figure 3). The old stem (primary) color of the Kediri accession is browner than the Cipanas accession. It has fewer round white spots than the Cipanas accession, which has more white spots.

The physiological characteristics between the two accessions did not show significant differences. Kediri and Cipanas accessions had the same chlorophyll a, b, and total chlorophyll (Table 2). The two accessions also exhibited comparable levels of anthocyanin and carotenoid contents. The number of stomata, stomata density, trichomes, and density of trichomes on the leaves of the Kediri accession and Cipanas accession from the two accessions showed no significant difference (Figure 4). At the same time, it can be described that the Cipanas accession has a larger stomata, stomata density, trichomes, and density of trichomes than the Kediri accession (Table 3).

Both accessions have different flower morphology at each development stage (Figure 5). The Kediri accession has a greater weight, length, and number of petals than the Cipanas accession (Table 4). The flowering stage influences the size of the *Cananga* flower, namely its weight, length, and number of petals. During observation, both accessions did not produce fruit.

The flowering stage significantly influences the number of trichomes found in the flowers of the Kediri and Cipanas accession *Cananga* (Table 5). The number of flower trichomes in the Cipanas accession was more than in the Kediri accession. The highest number of *Cananga* flower trichomes was at the initial flowering stage, and then the number decreased until the end. The *Cananga* trichomes of Cipanas and Kediri accessions are in Figure 6.

Table 1. Characteristics of leaves of two *Cananga* accessions

Variable	Accession	
	Kediri	**Cipanas**
Leaf color	Green	Green
Leaf length (cm)	16.03±1.5	14.5±1.2
Leaf width (cm)	7.61±0.8	6.74±0.5
Petiole color	Brownish red	Green
Leaf's central vein color	Brownish green	Green
Branch color	Brownish red	Green
Stem color	Dark brown	Light brown

Table 2. Leaf pigment analysis of two *Cananga* accessions

Accessions	Pigments (mg/g)				
	Chlorophyll *a*	**Chlorophyll *b***	**Chlorophyll total**	**Anthocyanin**	**Carotenoid**
Kediri	1.641±0.20	0.586±0.09	2.227±0.29	0.433±0.09	0.633±0.06
Cipanas	1.849±0.09	0.662±0.04	2.511±0.13	0.538±0.24	0.636±0.04
t-test	ns	ns	ns	ns	ns

Notes: ns: not significant

Table 3. Stomata and leaf trichomes of two *Cananga* accessions

Accessions	Number of stomata	Stomata density (/mm^2)	Number of trichomes	Trichome density (/mm^2)
Kediri	56.3±5.68	295.91±29.01	2.6±0.5	6.8±2.9
Cipanas	58.3±6.80	301.83±34.72	3.6±1.1	8.5±5.8
t-test	ns	ns	ns	ns

Notes: tn: not significant

Table 4. Weight, petal length, and number of flower petals

Variable	Flower morphological characteristics		
	Weight per flower (g)	Petal length (cm)	Number of petals
Accessions			
Kediri	1.99±1.2a	4.90±2.1a	13.59±1.1a
Cipanas	1.05±0.5b	4.75±2.4b	5.96±0.3b
Flowering stage			
Initial flowering	0.36±1.11e	1.67±0.2e	9.08±3.7b
25% full flowering	0.80±1.1d	3.00±0.3d	9.60±3.9ab
50% full flowering	1.41±1.07c	4.98±0.1c	10.10±4.3a
75% full flowering	2.24±1.17b	6.62±0.18b	9.60±4.2a
End-flowering	2.79±1.12a	7.85±0.15a	10.14±4.1a

Notes: Numbers followed by the same letter in the same column were not significantly different in the HSD test

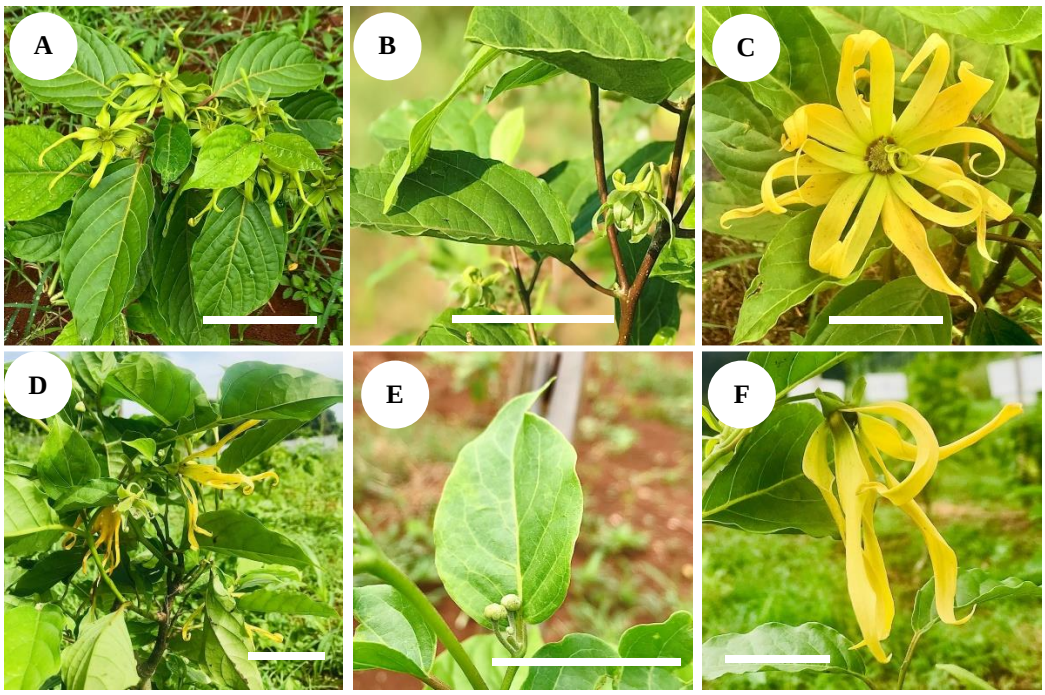

Figure 1. A-C. Kediri accession: A. Plant, B. Leaves, C. Flowers, D-F. Cipanas Accession: D. Plant, E. Leaves, F. Flowers. Bar = 5 cm

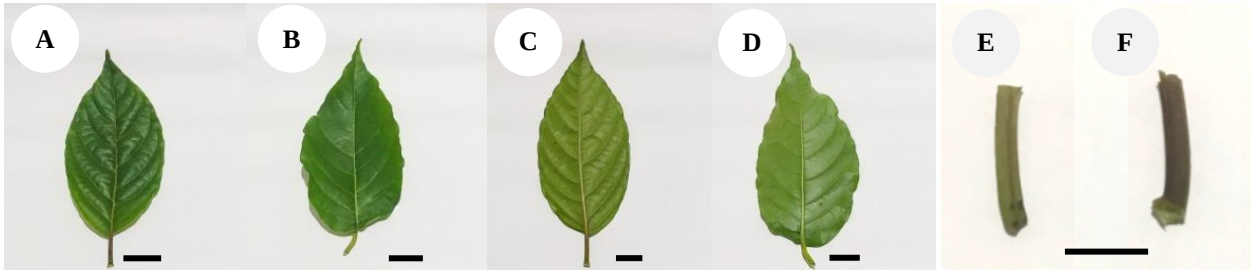

Figure 2. Upper leaves: A. Accession of Kediri, B. Accession of Cipanas. Lower leaves: C. Kediri accession; D. Cipanas accession; petiole: E. Cipanas accession; F. Kediri accession. Bar: 2 cm

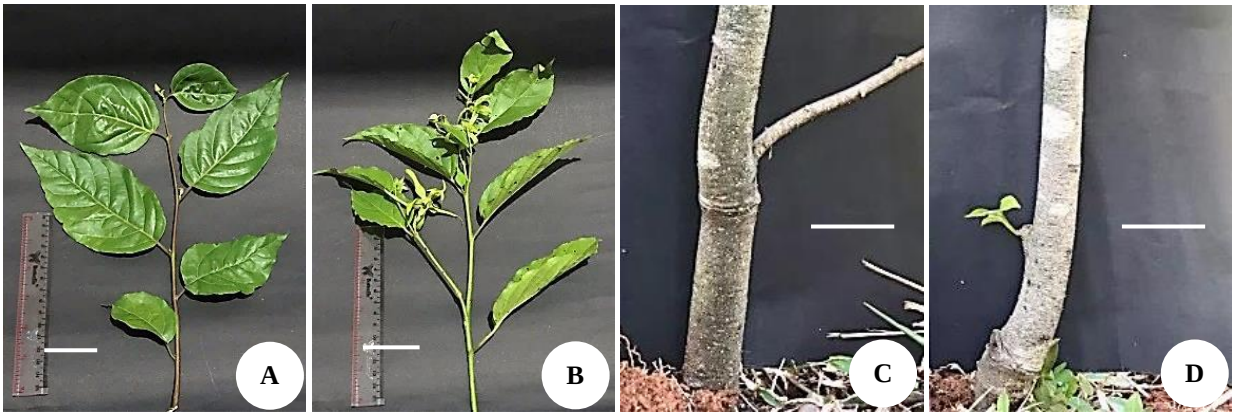

Figure 3. A. Branch of the Kediri accession, B. Branch of the Cipanas accession, C. Stem of the Kediri accession, D. Stem of the Cipanas accession. Bar = 5 cm

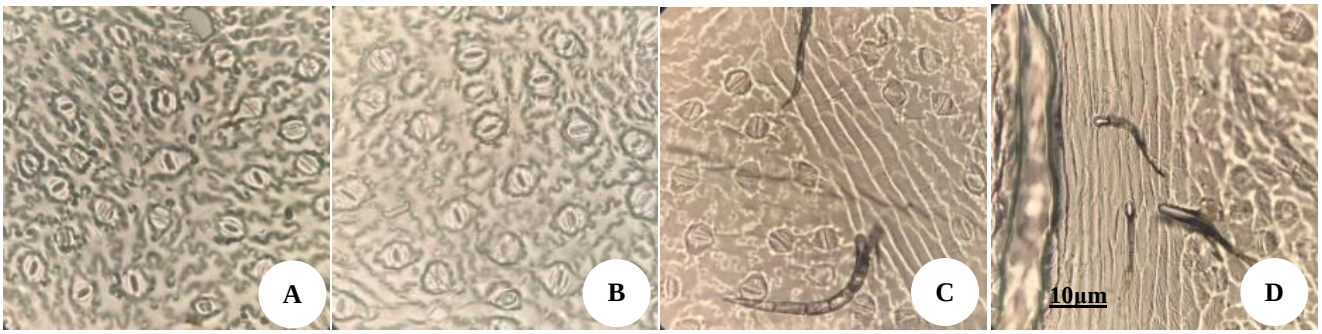

Figure 4. A. Stomata of Kediri accession, B. Stomata of Cipanas accession, C. Trichome of Kediri accession, D. Trichome of Cipanas accession

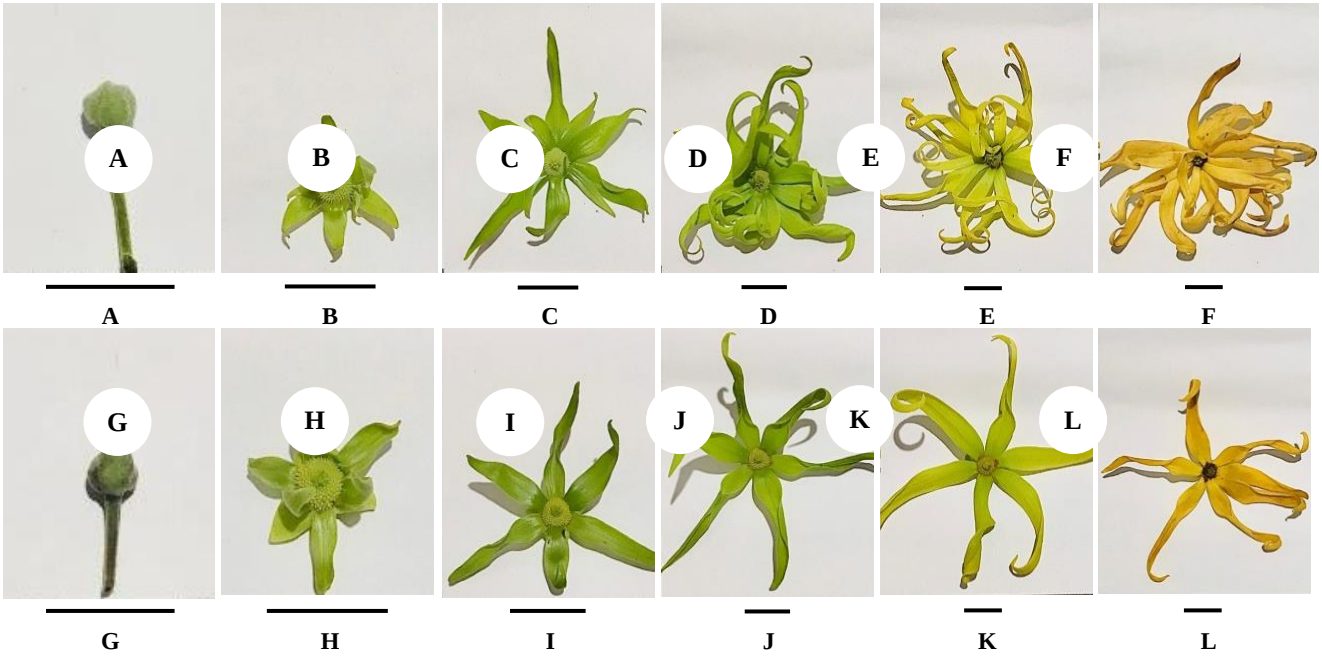

Figure 5. Kediri accession: A. Buds, B. Beginning of flowering, C. 25% full flowering, D. 50% full flowering, E. 75% full flowering, F. End-flowering. Cipanas Accession: G. Buds, H. Beginning of flowering, I. 25% full flowering, J. 50% full flowering, K. 75% full flowering, L. End-flowering. A and G. Bar = 1 cm, B-F and H-L. Bar = 2 cm

Moreover, flower development is also marked by changes in color (Table 6). The color in the initial flowering stage is green and will turn yellow as it progresses until it reaches the complete development stage. The color of *Cananga* lowers in the initial flowering stage is 5GY 5/4 with a light olive-green. Furthermore, the color intensity is the same for both *Cananga* up to the 50% full flowering stage, namely 5GY 6/6 with a moderate yellow-green. Next, the color difference between the two accessions occurs at the 75% full flowering stage, with a value of 2.5GY 5/6 with a moderate yellow-green for the Kediri accession and 2.5GY 7/8 with a strong yellow-green for the Cipanas. At the end of the flowering stage, both types of *Cananga* have the same color, with a value of 5Y 7/8 with a yellow. Therefore, the flower color of both accessions in the initial flowering stage, 25% full flowering, 50% full flowering, and the end flowering stage were the same.

The flower fragrance level was changed in the development stage. The initial flowering stage is that up to 25% of full flowers are not fragrant. As the flower develops, the fragrance of the flower increases, with the highest level of fragrance occurring in the end flower stage. The 50% flowering stage of the two accessions started to produce a slightly fragrant aroma, with a score of 2.5 and 2.8. It continues to increase until it becomes very fragrant in the end flowering stage at score 4.0 (Table 6).

Phenology of *Cananga*

The flowering phenology of *Cananga* for the two accessions is shown in Table 7. The Kediri accession has a flowering period of 35 days. In contrast, the Cipanas accession has a flowering period of 25 days. Based on the accumulated temperature, the flowering stage of both types of *Cananga* has a heat unit value of 591°CD and 432°CD.

***Cananga* essential oil**

The essential oil of *Cananga* flowers from Kediri and Cipanas in various flowering stages are presented in Table 8. The initial flowering stage produced the lowest oil content value of 0.15% in the Kediri accession and 0.20% in the Cipanas accession. The highest content of essential oils was produced in the end flowering stage at 0.61% for the Kediri accession, while the Cipanas accession was at the 50% flowering stage with an essential oil content of 0.92%.

The compound content identified in *Cananga* essential oil using the GCMS method is presented in Table 9. There were 20 compounds were identified from the two *Cananga* accessions and contained various compounds. The averaged highest metabolite compounds in the essential oil of the Kediri accession *Cananga* are linalool, caryophyllene, β-Copaene, and benzyl benzoate. Meanwhile, the Cipanas accession's averaged most abundant metabolite compounds were linalool, caryophyllene, α-Bergamotene, β-Copaene, trans-farnesol, and benzyl benzoate.

Table 5. Trichomes on *Cananga* flower petals of Kediri and Cipanas accessions

Flowering stage	Number of flower petal trichomes	
	Kediri	Cipanas
Initial flowering	41.6±4.7a	61.6±3.5a
25% full flowering	20.3±2.5b	41.6±7.5b
50% full flowering	15.3±1.5bc	40.3±5.8b
75% full flowering	13.3±1.5c	32.6±2.5bc
End-flowering	13.0±4.3c	30.6±15c

Notes: Numbers followed by the same letter in the same column are not significantly different, with an error rate of 5%

Table 6. Comparison of color and fragrance of *Cananga* flowers from Kediri and Cipanas accessions

Flowering stage	Kediri		Cipanas	
	Color	Fragrance	Color	Fragrance
Initial flowering	5GY 5/4	1.0	5GY 5/4	1.0
25% full flowering	5GY 5/6	1.1	5GY 5/6	1.4
50% full flowering	5GY 6/6	2.5	5GY 6/6	2.8
75% full flowering	2.5GY 5/6	3.6	2.5GY 7/8	3.8
End flowering	5Y 7/8	4.0	5Y 7/8	4.0

Notes: Color: GY: green-yellow; Y: yellow. Color characters refer to the Munsell Plant Tissue Color Book

Table 7. Phenology of *Cananga* flowering in Kediri and Cipanas accessions

Flowering stages	Kediri		Cipanas	
	Times (days)	Heat unit (°CD)	Times (days)	Heat unit (°CD)
Buds - initial flowering	10	174	8	136
initial flowering - 25% full flowering	8	137	6	102
25% full flowering - 50% full flowering	7	119	5	86
50% full flowering - 75% full flowering	6	93	3	54
75% full flowering - End-flowering	4	68	3	53
Total	35	591	25	432

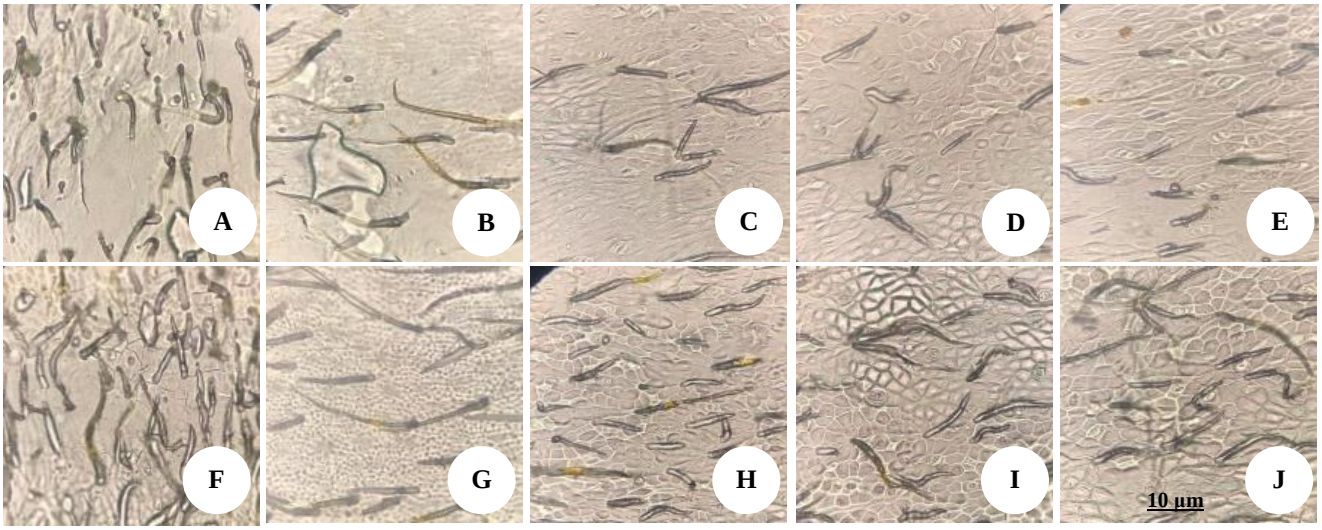

Figure 6. Petal trichomes of the Kediri accession: A. Beginning of flowering, B. 25% full flowering, C. 50% full flowering, D. 75% full flowering, E. End-flowering. Flower surface of Cipanas accession: F. Beginning of flowering, G. 25% full flowering, H. 50% full flowering, I. 75% full flowering, J. End-flowering

Table 8. Results of *Cananga* flower oil extraction at various flowering stages

Accession	Flowering stages	Flower weight (g)	Flower moisture content on a wet basis (%)	Oil volume (mL)	Content of essential oil (%)
Kediri	Initial flowering	237.00±38.2	19.10±0.7	0.35±0.1	0.15±0.01
	25% full flowering	211.90±42.0	22.00±3.0	1.00±0.1	0.47±0.03
	50% full flowering	230.15±18.9	22.16±0.9	1.13±0.7	0.51±0.36
	75% full flowering	238.33±32.5	23.49±1.1	1.37±0.1	0.58±0.05
	End flowering	225.33±34.5	26.43±1.7	1.43±0.8	0.61±0.28
Cipanas	Initial flowering	223.15±21.4	20.79±1.2	0.45±0.1	0.20±0.01
	25% full flowering	164.00±56.6	22.05±1.2	0.95±0.6	0.54±0.20
	50% full flowering	261.60±11.9	23.14±1.5	2.40±0.1	0.92±0.01
	75% full flowering	244.33±31.9	27.34±0.6	1.97±0.9	0.78±0.27
	End flowering	163.23±43.8	27.89±1.0	1.43±0.4	0.88±0.07

Table 9. *Cananga* essential oil profile

Retention time	Chemical compound	Abundance (%)					
		Kediri			Cipanas		
		50% full flowering	75% full flowering	End flowering	50% full flowering	75% full flowering	End flowering
3.388	α-Thujene	1.09	1.14	1.04	1.00	1.20	1.15
5.769	β-Myrcene	1.16	1.17	-	1.08	1.04	1.01
7.783	trans-β-Ocimene	2.45	2.65	2.26	2.50	2.35	2.30
8.338	β-Ocimene	3.15	3.37	2.86	3.23	3.01	2.92
16.131	Benzene, 1-methoxy-4-methyl-	1.52	2.14	1.69	1.52	1.73	1.68
21.953	Linalool	9.68	9.40	9.45	18.74	16.51	9.70
23.852	Caryophyllene	13.58	11.71	15.92	15.08	16.51	16.00
27.541	Humulane	3.85	3.17	4.71	4.68	5.09	4.71
29.492	α-Bergamotene	6.89	-	-	5.45	6.65	9.68
29.502	Cis-trans-α-Farnesene	-	11.28	11.12	-	-	-
29.668	β-Copaene	12.82	12.23	14.07	11.38	11.67	12.59
32.527	α-Farnesene	5.92	7.62	7.16	4.64	5.77	6.63
35.490	3,4-Dimethoxytoluene	2.50	3.05	2.59	3.43	3.69	2.60
45.708	Methyleugenol	1.70	1.29	1.41	2.89	2.09	1.38
49.821	α-cadinol	3.38	2.61	1.81	-	-	2.06
49.852	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)	-	-	-	2.12	1.81	-
50.283	(2E,6E)-3,7,11-Trimethyldodeca-2.6.10-trienyl propionate	-	-	-	-	-	1.19
51.549	All-trans-Farnesol acetate	14.24	-	1.07	-	-	-
51.544	Trans-Farnesol	-	11.52	9.21	9.57	8.20	10.99
56.924	Benzyl Benzoate	7.01	7.39	6.36	5.13	5.85	7.29

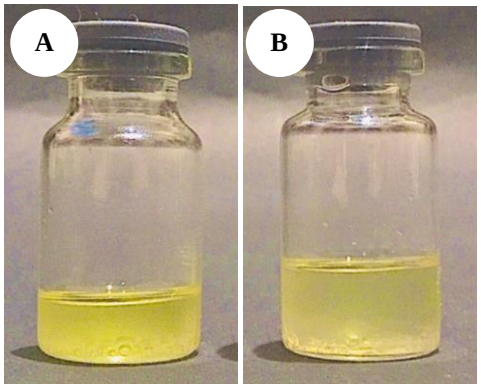

Figure 7. *Cananga* essential oil in the end flowering stage A. Kediri accession. B. Cipanas accession

Moreover, several unique compounds are contained only in Kediri or Cipanas accession. Special compounds that only the Kediri accession has been Cis-trans-α-Farnesene and all-trans-Farnesol acetate. Meanwhile, the compounds only possessed by the Cipanas accession are Benzene 1,2,3-tri methoxy-5-(2-propenyl) and (2E,6E)-3,7,11-Trimethyldodeca-2.6.10-trienyl propionate. In the Kediri accession, the highest compound content was all-trans-Farnesol acetate 14.24% 50% full flowering stage, 12.23% β-Copaene 75% full flowering, and 15.92% Caryophyllene end flowering stage. Meanwhile, in the Cipanas accession, the highest compound content was 18.74% linalool in the 50% full flowering, 16.51% linalool in the 75% full flowering, and 16.51% caryophyllene in the end flowering stage.

Discussion

Cananga is a complete or perfect flower with petals, sepals, pistils, and stamens (Nurhayani et al. 2019). The morphology of the two *Cananga* accessions observed showed differences in the leaf and stem characteristics, including leaf length, leaf width, petiole color, leaf's central vein color, and branch color. The flower color of both accessions in the initial flowering stage, 25% flowering, 50% flowering, and the end stage, were the same. The initial flowering stage has the lowest weight, length, and number of petals and increases in size with the development of the flowering stage; the end flowering stage has the largest flower size. The *Cananga* is usually used as fresh flowers, considering the level of its fragrance. Therefore, the flower development stage, where the flowers begin to emit a fragrant aroma, is the appropriate stage for harvesting for fresh use.

There are two types of trichomes, namely glandular and non-glandular. Glandular trichomes have a bubbly shape containing secondary metabolites, while non-glandular trichomes have branched or unbranched unicellular or multicellular forms (Juhari et al. 2014). Some trichome genes can be involved in flower biosynthesis to produce odors, taste, color, and secondary metabolite compounds (Li et al. 2020). Trichomes of *Cananga* flower petals are also glandular, which secrete *Cananga* essential oil, allowing the flower petals to release their fragrance.

In addition, the quantity and quality of essential oils are influenced by several factors, including differences in soil quality, temperature (Sestelo and Carrillo 2020), plant origin, and flower development (Hassiotis et al. 2014). The flowering period of the Cipanas accession is 25 days, and the Kediri accession is 35 days. It was also reported by Ramadhani and Salamah (2021) that the development of *Cananga* flowers can be categorized into several stages, starting from bud to mature flower, which takes around 35 days. The Kediri accession requires a heat unit of 591°CD, while the Cipanas accession requires 432°CD in the flower development process from flower buds to the end of flowering stages. Heat unit is used to predict growth stage and physiological response by the temperature accumulation. The heat unit can influence the molecular aspects of flower induction (Parthasarathi et al. 2013).

The average minimum and maximum temperature in the research field ranges from 23-32°C. *Cananga* grows in warmer areas, which allows the flower growth period to occur more quickly. *Cananga odorata* thrives in more humid lowland tropical areas with an average annual temperature of 21-27°C (Yusuf and Sinohin 1999). Forest cloves' heat units and phenology are important in planning cultivation with similar climatic conditions to project the right harvest time (Kamsurya et al. 2022). The flowering and fruiting period in plants is a natural period that is seasonal and is often influenced by temperature. In addition, Annonaceae plant species in subtropical areas have an earlier flowering and fruiting period than those in tropical areas (Lestari 2020).

Several factors, including the flower growth stage, can influence the essential oil contents. In all flowering stages, the essential oil content from the *Cananga* flowers of the Cipanas accession was higher than from the Kediri. The highest essential oil content is 0.92%, produced by Cipanas accession at the 50% full flowering stage. The previous research stated that the oil content of *Cananga* produces an oil content of around 1.27-2.3% (Mahfud et al. 2017; Uday et al. 2022). The optimal stage to get the higher content of *Cananga* essential oil is at the flowering stage starts to turn yellow with a purplish-red heart (Chakira et al. 2022). Hydrodistillation produced more qualified *Cananga* oil regarding oxygenated compounds and ester content than steam-water distillation (Oktavianawati et al. 2022). Therefore, the best peppermint essential oil results are produced during the first harvest with 50% chemical and nano-chelated fertilizers (Ostadi et al. 2020). The best time to harvest *Thymus capitatus* (L.) Hoffmanns. & Link for phenol content is during or immediately before the full bloom (Casiglia et al. 2015). Lemongrass oil content increases with a larger surface area containing more oil content than leaves with a smaller size (Akhihiero et al. 2013). The growth time and type of *B. balsamifera* plant organs influence the composition and content of essential oil produced. The essential oil level in the young leaves was the highest, followed by mature and senescent leaves, with 44 compounds identified (Yuan et al. 2016).

The physical quality standards for *Cananga* essential oil are based on the Indonesia National Standard (SNI 06-3949-1995), including color, refractive index, specific

gravity, optical rotation, ester number, solubility in ethanol, and steam distillation residue. According to SNI, the physical characteristic of *Cananga* flower oil is light yellow, and this study revealed that the essential oil from various stages of flower development is light yellow (Figure 7). This means that based on the color of the oil, both Kediri and Cipanas accessions produce *Cananga* essential oils that comply with SNI. Moreover, all oils from various stages of flower development produce the flower's characteristic fragrant aroma.

The morphology of *Cananga* leaves from Kediri and Cipanas accessions has differences in the leaf stalk, branch color, and stem color. The flower morphology also differs between these two accessions. The Kediri accession flower has a 13 number of petals is higher and longer in size, and the weight per flower is heavier than the Cipanas accession at all flowering stages. The Kediri accession's flowering stage lasts 35 days, while the Cipanas accession takes 25 days. The highest essential oil content of the Cipanas accession was in the 50% full flowering stage of 0.92% and decreased until the end flowering stage. The Kediri accession produced the highest essential oil content at the end of the flowering stage, reaching 0.63%. Thus, the Cipanas accession flowers should be harvested earlier than the Kediri accession. However, the flower weight of Kediri accessions is heavier at that same stage.

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