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Genetic stability of melon (*Cucumis melo* L. cv. Meloni) based on inter-simple sequence repeat and phenotypic characteristics

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Abstract. *Yusuf AF, Wibowo WA, Daryono BS. 2022. Genetic stability of melon (*Cucumis melo *L. cv. Meloni) based on inter-simple sequence repeat and phenotypic characteristics. Biodiversitas 23: 3042-3049.* A new cultivar must have a character that follows the official criteria of distinctness, uniformity, and stability (DUS) as required for the grant of Plant Breeder Rights and official cultivar registration. Meloni is a new cultivar resulting from plant breeding by the Genetics and Breeding Laboratory of the Faculty of Biology, Universitas Gadjah Mada. Legality is needed in the form of plant variety protection (PVT) to protect the intellectual rights of researchers. Therefore, this study aims to identify morphological characters according to official criteria and validate molecular feelings by using molecular markers Inter-Simple Sequence Repeat. The results of morphological characterization showed that the distinctive character of the Meloni cultivar lies in the oval shape of the fruit, the skin of the fruit is creamy with a smooth texture without the net, and the flesh of the fruit is orange with a sweet taste. The differences in morphological characters of the Meloni cultivar cultivated at two locations differ in fruit weight, flesh thickness, fruit shelf life, and harvest age. Molecular identification resulted in a low similarity in the phenetic relationship, namely 35% against Sonya cultivars and 51% against Kirani and Kinanti cultivars. The Meloni cultivar's stability and uniformity analysis between the two locations (Bandung, West Java, and Sleman, Yogyakarta) yielded 76%. The difference in cultivation locations did not affect the level of stability and genetic uniformity of the Meloni cultivar.

Keywords: *Cucumis melo* L. cv. Meloni, ISSRs markers, phenotype, genotype, DUS (distinctness, uniformity, and stability)

INTRODUCTION

Melon (*Cucumis melo* L.) is a flora member of the Cucurbitaceae family, which has abundant species diversity and is commonly developed by the Indonesian people as a horticultural commodity. According to one opinion, the origin of melon comes from North Africa, a relatively dry and hot area. However, some statements also state that melons originate from the Mediterranean area. The main distribution of melons started in Europe and the Middle East. Over time, the spread of melons expanded to include tropical and subtropical climates in Indonesia (Daryono and Maryanto 2017).

Plant breeding is a form of improvisation of agricultural products such as melons. The application of plant breeding is based on community needs and productive agriculture (Ewing et al. 2019). The application of plant breeding science to produce or maintain the unique character of a type of plant inherited by the next generation. The combination of textures between these crosses gives rise to a new product, namely plant varieties (Zaidi et al. 2019).

Melon cultivar Meloni is one of the plant breeding results by the Genetics Laboratory and breeding of the Faculty of Biology, Universitas Gadjah Mada, motivated by Indonesian melon farmers' dependence on premium imported seeds. Meloni cultivar results from a cross between female parent Sun Lady 3 (SL-3), the third generation of Sun Lady melon originating from Taiwan, and male parent PI 371795 originating from India. The main characteristics of the melon are bright orange flesh color (orange group RHS 26 D), sweet taste with sugar content between 7-16 Brix, moderate aroma, and ability to last for 11-15 days at room temperature. The harvest period ranges from 58-64 days after planting with suitable adaptation areas in the lowlands (Daryono and Maryanto 2017).

A new plant variety requires testing before being released to the public. The test is a series of tests for stability, uniformity, uniqueness, and novelty compared to other commercial types. In addition, to protect the intellectual rights of researchers as developers of new cultivars, legality in the form of plant variety protection (PVT) is required, which is confirmed through morphological and molecular characterization. Therefore, the state has the right to protect plant varieties resulting from plant breeding, called the Plant Variety Protection Rights (PVT), regulated in the Republic of Indonesia Law Number 29 of 2000.

The characterization was based on morphological and molecular determination based on the molecular marker Inter-Simple Sequence Repeat (ISSR). ISSR is a molecular marker based on polymerase chain reaction (PCR) amplification in regions in the genome flanked by microsatellite sequences. The PCR amplification process in this region using a single primer produces an amplification product used as a multilocus marker system to study genetic variation (Marwal and Gaul 2020).

Meloni is a new cultivar that can be Indonesia's premium melon. This study aims to determine genetic variation, stability, and uniformity based on the phenotypic

and molecular characteristics of Meloni cultivars cultivated at different locations. In addition, Meloni, as a new cultivar, requires legality in plant variety protection (PVT), which is confirmed through morphological and molecular characterization with ISSR markers. This data is also an essential component in identifying and conserving Indonesia's biodiversity.

MATERIALS AND METHODS

Study area

This research was conducted from July 2019 to March 2020. Meloni cultivars were planted in Pangalengan, Bandung, West Java and Mutihan, Sleman, Yogyakarta, Indonesia. Meanwhile, the analysis of phenotypic and molecular characters was carried out at the Genetics and Breeding Laboratory, Faculty of Biology, Gadjah Mada University. The commercial melon cultivars used as comparisons were Kirani, Kinanti, and Sonya.

Procedures

Phenotypic characterization and sample collection

The observed phenotypic characters include characters that indicate the morphological and agronomic characteristics of the plant. Observation of qualitative characters in the form of color using RHS color chart and quantitative observation using ruler and Medline. Sample collection by taking samples of leaves 3-4 weeks old and fruit. The cut leaves and fruit were put in a plastic icebox and stored in the freezer at 20ºC.

DNA extraction

DNA extraction using the Nucleon Phytopure Kit followed the previous research about melons. The destruction process by grinding 0.5 g of leaf samples with a pestle and mortar until smooth with 200 µL of Phytopure 1 reagent. The mixture was added with 300 µL of Phytopure 1 reagent and 200 µLof Phytopure 2 reagent incubated at 65ºC for 20 minutes. After completion, the mixture was added with 400 µL of cold chloroform and 20 µL of Phytopure Resin and then centrifuged at 1300 rpm for 10 minutes. The supernatant obtained was transferred to a new tube, and cold isopropanol was added in a ratio of 1:1. The mixture was inverted and centrifuged to produce pellets that were DNA precipitates. Three times purified 70% ethanol and added 50 µL 1X buffer TE. The results of the isolation were saved at a temperature of -20ºC. A quantitative test of DNA amplification results using Nanodrop UV-Vis (NanoVue 4282 V2.0.4 Beckman) to determine the purity and concentration of isolated DNA.

DNA amplification by PCR-ISSR

DNA amplification using a DNA PCR Kit (2x My Taq HS Red Mix Bioline) and ISSR primers UBC 807, UBC 808, UBC 810, UBC 812, and UBC 825, which have been described in the Table 1. PCR Kit Reagents Bioline 12,5 µL, 8.5 µL of sterile ddH2O, 2 µL of ISSR primer, and 2 L of template DNA were inserted into a microtube homogenized by a vortex and then amplified using a BOECO Thermal cycler PCR. The amplification results were analyzed qualitatively using agarose gel electrophoresis and visualized with GelDoc.

Data analysis

Quantitative data was analyzed using ANOVA testing with SPSS software with one factor at a significance level of 1% to calculate the range, mean, and variance. Further analysis of quantitative character tests used the LSD test and Duncan test. Qualitative data were analyzed descriptively. The overall phenotypic and molecular character data were converted into a 0-1 matrix to construct a dendrogram of phenetic relationships using the MVSP 3.1 program and Microsoft Excel 365 student edition.

RESULTS AND DISCUSSION

Distinction characteristics of Meloni cultivar

Melon has characteristics that distinguish it from other species and the taxa, namely cultivars. Each melon cultivar has specific characteristics that distinguish it from other cultivars, provided that the character is passed on to the next generation. Analysis of genetic diversity based on phenotypic characters can show differences in the melon cultivar Meloni and its comparison. The comparison commercial melons Kirani and Kinanti are classified as inodorus melons with smooth skin without a net. In contrast, Sonya is classified as melon reticulatus because there is a net on the fruit surface.

The Meloni cultivar has an ovoid shape, while the comparison melon cultivar has a globular and ovate shape (Figure 1). Meloni cultivars do not smell fragrant when ripe, so they are included in non-aromatic melons. The two also do not have a fragrant character because the Meloni cultivar results from a cross between SL-3 and PI 371795. Based on the data shown in Table 2, the Meloni cultivar has an average weight of 797.6±168.9 g with a sweetness level ranging from 8-10 Brix. The fruit is creamy white (YWG 158C) with orange flesh (OG 24B). The shelf life of the fruit reaches 17 days after harvest.

Table 1. ISSR primers with nucleotide sequences and nitrogenous bases

Primers	Nucleotide sequence (5' – 3')	Nitrogen base	Annealing temperature (ºC)
UBC 807	AGAGAGAGAGAGAGAGT	17	50
UBC 808	AGAGAGAGAGAGAGAGC	17	50
UBC 810	GAGAGAGAGAGAGAGAT	17	46.2
UBC 812	GAGAGAGAGAGAGAGAA	17	46.2
UBC 825	ACACACACACACACACT	17	40

Table 2. The phenotypic character of melon cv Meloni and comparison with commercial cultivars

Phenotype character	Cultivar			
	Meloni	**Kinanti****	**Kirani****	**Sonya****
Plant type	Annual	Annual	Annual	Annual
Habitus	Herbaceous	Herbaceous	Herbaceous	Herbaceous
Leaf-blade shape	Triangularis	Triangularis	Triangularis	Triangularis
Stem shape	Silindris	Silindris	Silindris	Silindris
Flower shape	Rotate	Rotate	Rotate	Rotate
Fruit shape*	Ovaloid	Ovate	Ovate	Globulate
Seed shape	Tapered ellipse	Tapered ellipse	Tapered ellipse	Tapered ellipse
Fruit Aroma	Non-aromatic	Non-aromatic	Non-aromatic	Non-aromatic
Skin fruit type*	Inodorus	Inodorus	Inodorus	Reticulatus
Leaf-blade color*	NN137A	139A	139A	GGN 137C
Stem color*	144D	139C	138C	NN137C
Petal color*	143C	143C	146C	144C
Crown color*	9A	9A	7A	9A
Stamen color*	9A	6D	2A	2C
Pistil color*	1A	1C	2C	144A
Seed color*	CYG 161A	GYG 161A	GYG 161C	GOG 163D
Skin fruit color*	YWG 158C	YG 11A	WG 28C	YGG N 137A
Flesh fruit color*	OG 24B	OG 24C	OG 24B	OG 24B
Sweetness level*	11-12 ^{o}brix	10-11 ^{o}brix	9-12 ^{o}brix	8-10 ^{o}brix
Flowering age of ♂ flower*	35 DAP	38 DAP	38 DAP	30 DAP
Flowering age of ♀ flower*	37 DAP	40 DAP	40 DAP	33 DAP
Harvest age*	80 DAP	83 DAP	83 DAP	70 DAP
Storability*	17 DAH	17 DAH	25 DAH	27 DAH
Leaf-blade area (cm^2)*	470.9 ± 48.0^{a}	475.9 ± 68.3^{b}	474.9 ± 35.3^{b}	522.3 ± 38.7^{c}
Terminal cup (cm)*	6.5 ± 0.9^{a}	6.6 ± 0.6^{a}	7.8 ± 0.6^{b}	8.2 ± 0.8^{b}
Length of petiole (cm)*	15.5 ± 1.2^{a}	16.5 ± 2.1^{b}	15.0 ± 1.1^{a}	19.1 ± 1.2^{c}
Diameter of stem (cm)*	1.0 ± 0.0^{a}	1.1 ± 0.1^{a}	1.0 ± 0.1^{ab}	1.1 ± 0.1^{b}
Number of lateral branches*	19.9 ± 2.8^{a}	19.5 ± 2.6^{a}	20.3 ± 1.7^{a}	23.3 ± 1.2^{b}
Diameter of fruit (cm)*	9.3 ± 0.5^{b}	9.50 ± 0.39^{a}	9.67 ± 0.26^{ab}	13.03 ± 2.98^{c}
Weight of fruit (g)*	797.6 ± 168.9^{b}	705 ± 108.8^{ab}	653.8 ± 63.2^{a}	1157.8 ± 110^{c}
Seed's area (cm^2)*	0.5 ± 0.0^{c}	0.52 ± 0.02^{c}	0.44 ± 0.02^{a}	0.48 ± 0.03^{b}

Note: *different characters; Lowercase letters indicate differences in subsets at a significance level of 1%; (±) indicates the standard deviation value. **published data (Yusuf and Daryono 2021)

Based on ISSR molecular markers (Table 3), the number of DNA fragments amplified by 5 ISSR primers (UBC 807, UBC 808, UBC 810, UBC 812, and UBC 825) was 29 DNA fragments with 12 polymorphic DNA fragments. The lowest percentage of polymorphism was produced by primer UBC 807 with 20%. The highest percentage was produced by primer UBC 808 with a value of 100%. Primer UBC 808 is considered to be used as a molecular marker for genetic variation tests in melon because it produces a polymorphism percentage above 50%. The high level of polymorphism indicates the high genetic variation between melon cultivars.

The phenetic relationship of Meloni cultivar and comparative cultivar

Reconstruction of dendrogram and similarity matrix using OTU (Operational Taxonomic Unit) combines phenotypic and molecular characters with the UPGMA method and Jaccard coefficient approach. The lowest similarity matrix is between Kinanti and Sonya cultivars, with a value of 0.32. Meanwhile, the highest similarity matrix is between Kirani and Kinanti cultivars, with a value of 0.51.

Based on Figure 2, the phenetic relationship dendrogram forms two main clusters. The first cluster consists of melon inodorus (winter melon), namely Meloni, Kinanti, and Kirani cultivars. The closest phenetic relationship is Kirani and Kinanti, with a similarity percentage of 51%. Meloni cultivars clustered at a similarity percentage of 46%. The second cluster is the Sonya cultivar with reticulatus type (netted melon). The percentage of similarity between the two clusters is 35%. The low similarity percentage indicates that the phenetic relationship between cultivars is low, and the genetic variation between cultivars is high.

Stability and Uniformity of Meloni cultivar at the two cultivation sites

Based on Table 5, the high average humidity in the lowlands can indicate higher fungal growth compared to the highlands. The planting method between the two different locations determines the length of time for cultivation and the shelf life of the fruit. Direct observations showed that plants grown in closed greenhouses were healthier and better than those grown in conventional screen houses.

The uniformity of melon fruit characters is shown in Table 6. The characters maintained at both cultivation locations were plant type, habitus, leaf shape, stem shape, flower shape, fruit shape, seed shape, fruit type, flesh texture, fruit skin type, dot color, and fruit color. Fruit weight, flesh thickness, fruit shelf life, and harvest age are different characteristics between the two cultivation sites. The color characters, when observed directly, are relatively the same. Although, on specific observations with the RHS color chart, different color complexes are obtained.

The total amplified DNA fragments based on the ISSR molecular marker were 22 fragments with 7 polymorphic loci (Table 7). Polymorphic DNA was only found in the primers UBC 812 and UBC 825, with the polymorphism percentage values of 60% and 67%, respectively. The average level of polymorphism in the five primers used was 25%. This value is relatively low (≤50%), which indicates the low genetic variation between Meloni cultivars. Therefore, location elevation did not affect the stability and uniformity of the melon cultivar Meloni.

The phenetic relationship between individuals of the Meloni cultivar at the two cultivation sites

The similarity matrix in this section is the result of analysis using phenotypic and molecular characters using the UPGMA method and the Jaccard coefficient approach. Based on Table 8, the highest similarity value (1.00) on all individual Meloni cultivars cultivated in Bandung, West Java. Conversely, the lowest similarity index value was found between individuals produced in Bandung, West Java, and one cultivated in Sleman, Yogyakarta (MLI.1.M), with a similarity value of 0.74. Overall, the value of similarity between individual Meloni cultivars cultivated at different locations is high.

Based on Figure 3, two main clusters were formed, showing the phenetic relationship between individual Meloni cultivars. The first cluster is the Melon cultivar group cultivated in Bandung, West Java, and all individuals (MLI.1.N, MLI.2.N, and MLI.3.N) have a similarity percentage of 100%.

The second cluster consisted of individual Meloni cultivars cultivated in Sleman Yogyakarta. MLI. 2.M and MLI.3.M were grouped with a similarity percentage value of 93%, then MLI.1.M with a similarity percentage of 89%. The similarity percentage between the two locations (Bandung, West Java, and Sleman, Yogyakarta) has 76%. These values indicate that the individual melon cultivar Meloni is uniform and has a close phenetic relationship. Differences in location and planting methods do not cause genetic changes to maintain the phenotypic character.

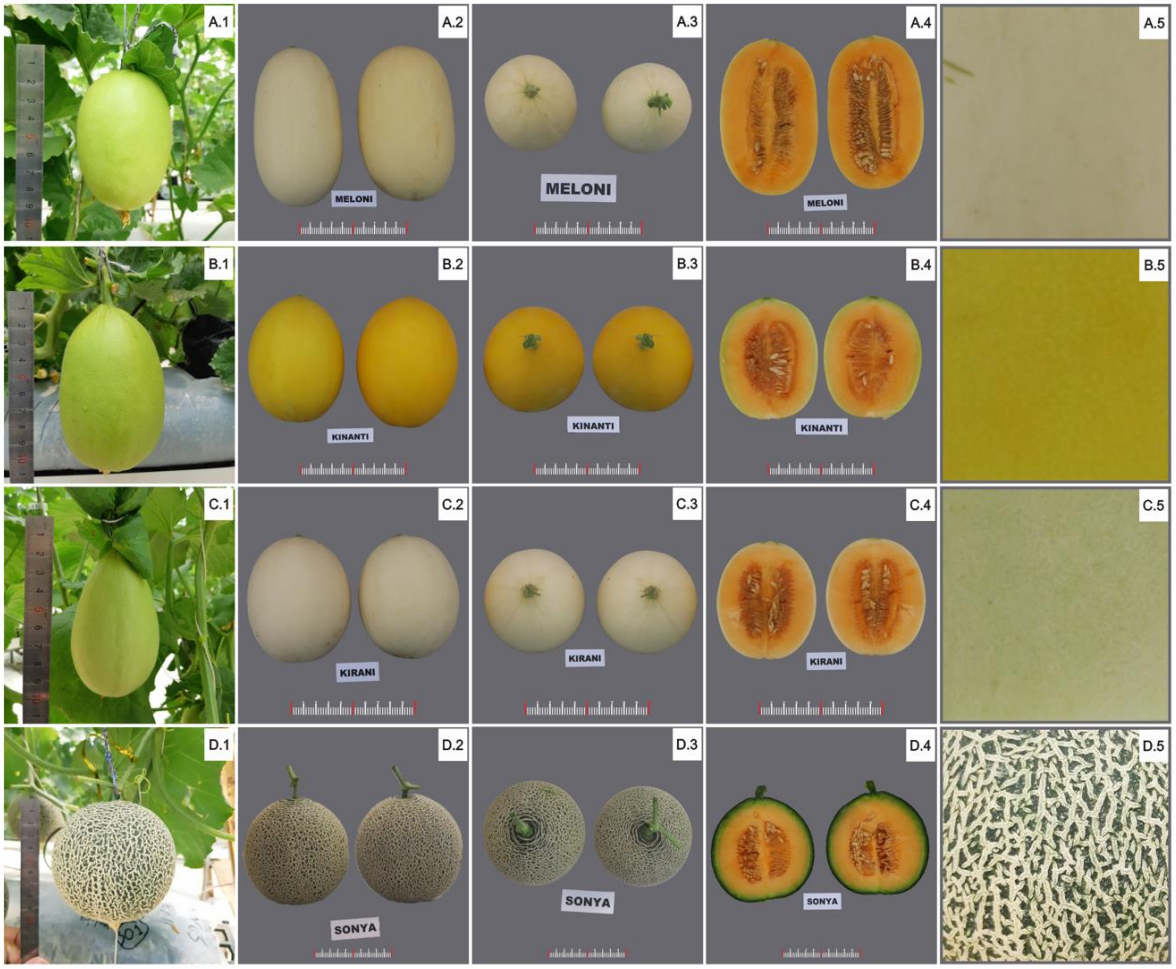

Figure 1. Morphological characteristics of the Meloni cultivar and comparable to another commercial melon. A: Meloni, B: Kinanti, C: Kirani, D: Sonya, 1: young fruit, 2: side view, 3: top view, 4: inside view of riped fruit, 5: dots

Table 3. Percentage of DNA polymorphism of Meloni cultivar and another comparable cultivar

ISSRs primer	Total amplified DNA	Polymorphic loci	Polymorphic rate (%)	Fragment size (bp)
UBC 807	5	1	20.0	332-1320
UBC 808	8	5	62.5	438-1485
UBC 810	4	1	25.0	645-1196
UBC 812	5	2	40.0	436-1320
UBC 825	7	3	42.9	512-1301
Average	5.8	2.4	37.9	-
Total	29	12	-	-

Table 4. Similarity matrix of Meloni cultivar and another comparable cultivar

	Meloni	Kinanti	Kirani	Sonya
Meloni	1,00			
Kinanti	0,50	1,00		
Kirani	0,42	0,51	1,00	
Sonya	0,38	0,32	0,35	1,00

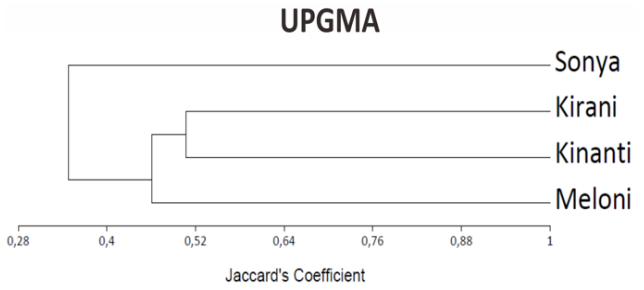

Figure 2. Phenetic relationship on individual Meloni cultivars based on UPGMA results with Jaccard coefficient approach

Discussion

Plant variety is one of the success factors in horticultural cultivation. It is related to ensuring the quality of varieties that will affect productivity, disease resistance, and quality of crop yields and reduce the risk of foul in cultivation. Plant variety (according to UU No. 12. 1992) is part of a species characterized by plant shape, growth, leaves, flowers, fruit, seeds, and other characteristics that can be distinguished in the same type. According to UU No. 29. 2000, plant variety is a group of plants of a type or species characterized by plant form, plant growth, leaves, flowers, fruit, seeds, and expression of genotypic characters or combinations of genotypes that can distinguish from the same type or species by at least one the defining characteristic and when reproduced it does not change.

In botanical terms, plant varieties are referred to as cultivars. The cultivar is an abbreviation of cultivated variety, a plant that farmers breed. The development of new cultivars is carried out by applying the science of plant breeding that can combine various superior characteristics. Diversity (variability) and mean plant population have an enormous impact on the success of plant breeding. According to the breeding target, success in conventional breeding depends on phenotypic and phenotypic variance expression. The stability of the new cultivars was demonstrated by the minimum coefficient of variation (CV) of single plant yields and controlled spatial heterogeneity (Fasoula et al. 2020). Parental selection increases the probability of forming superior cultivars because of the genetic diversity that makes it possible to select superior genotypes (Carneiro et al. 2021). The method of cultivar assembly by hybridization cannot be separated from Mendelian's Law I regarding the segregation of allele pairs during gamete formation and Mendelian II regarding alleles inherited by parents who will pair independently at fertilization (Van Dijk 2018). The main objective in the assembly of cultivars is the selection of superior plant cultivars or varieties with the desired traits by breeders with a level of uniformity and agronomic stability, and good adaptability (Breseghello and Coelho 2013). However, the release of a variety requires a series of tests to ensure that the new variety registered meets the requirements as a variety or cultivar.

The assessment of a new cultivar is usually carried out by the Ministry of Agriculture (Indonesia) or related institutions, which involves testing phenotypic characters. Phenotypic characters are external characters that can be observed (qualitatively) or measured (quantitatively) and influenced by genotype, epigenetic modification, and environmental factors. The phenotypic character is determined by the interactions of the proteins present in the cell. The protein is composed of amino acids with a particular sequence encoded by a gene (Nussinov et al. 2019). A genotype is a combination of alleles that an organism has for genes that are part of a particular genetic makeup of an organism that determines its phenotypic character (Hallgrimsson and Hall 2011). Therefore, changes in the phenotypic character are always influenced by the genotype that expresses it. So, genotype stability is crucial in the identification of new cultivars.

Table 5. Environmental conditions at the two cultivation sites

Location		**Sleman, Yogyakarta**	**Bandung, West Java**
Time		**Mey-June**	**July-October**
Altitude	*	115-meter asl	1432-meter asl
Latitude	*	7.80 S, 110.51 E	7.20 S, 107.55 E
Temperature	**	25.17 °C	23.27 °C
Humidity	**	79.52 %	66.86 %
Rainfall	**	0.5 mm	0.5 mm
Sun exposure	**	7.7 hour	7.7 hour
Condition	***	Semi-enclosed greenhouse. The temperature inside the screenhouse depends on the sun's intensity, but the soil acts as a heat sink.	Closed greenhouse with modern construction equipped with air circulation, automatic watering, and cooling area.

Note: *from earth.google.com; S: south; E: east; **The average climate parameter data is taken from the Meteorology, Climatology and Geophysics Agency (BMKG); ***The condition is the result of direct observation by researchers in the research area

Table 6. The stability of the morphological character of melon Meloni cultivars at the two cultivation sites

Characters	**Cultivation sites**	
	Bandung, West Java, Indonesia	**Sleman, D. I. Yogyakarta, Indonesia**
Plant type	Annual	Annual
Habitus	Herbaceous	Herbaceous
Leaf-blade shape	Triangularis	Triangularis
Stem shape	Silindris	Silindris
Flower shape	Rotate	Rotate
Fruit shape	Ovaloid	Ovaloid
Seed shape	Ellipse	Ellipse
Fruit shape	Ovaloid	Ovaloid
Fruit type	Inodorus	Inodorus
Flesh fruit texture	Crispy	Crispy
Fruit skin type	Smooth	Smooth
Dots color	White	White
Young fruit color	RHS 4D (Light Green)	RHS 4D (Light Green)
Ripe fruit color	RHS 158A (Creamy White)	RHS 158A (Creamy White)
Flesh fruit color	RHS 24B (Orange)	RHS 24D (Orange)
Sweetness level	9-11 ^{o}brix	10-11.5 ^{o}brix
fruit weight	797.58 ± 168.90 cm	747.69 ± 157.05 cm
Thick flesh fruit	2.43 ± 0.31 cm	1.73 ± 0.29 cm
Storability	17 DAH	9-10 DAH
Harvest age	80-85 DAP	53-58 DAP

A new cultivar must have a character that follows the official criteria of distinctness, uniformity, and stability (DUS) as required for granting Plant Breeding Rights and official cultivar registration (Korir et al. 2012). The distinctive character of the Meloni cultivar lies in the ovoid shape of the fruit, the skin of the fruit is creamy with a smooth texture without a net, and the flesh of the fruit is orange with a sweet taste. The ovoid fruit shape was a conserved character of the parent PI 371795 when a cross between melon SL-3 and PI 371795 resulted in the Meloni cultivar. Melon PI 371795 is a melon with an ovoid shape, green skin, yellow color, and a bitter fruit taste (Daryono and Maryanto 2017). While the broodstock SL-3 is a melon with an ovate shape with creamy skin, orange flesh, and a sweet taste (Ishak and Daryono 2020). The character of taste and color is then passed on to the Meloni cultivar. The combination of characters from the two parents resulted in Meloni cultivars having unique characteristics that were different from other commercial cultivars and met the requirements of a new cultivar.

However, two other requirements must be ensured in releasing plant varieties: stability and uniformity. Proof of stability and uniformity should be carried out through multi-season and multi-site tests. In this study, the multi-location test was based on differences in altitude, and the multi-season test was conducted in two different seasons. The different characters in the Meloni cultivar were in the level of sweetness, fruit weight, flesh thickness, fruit shelf life, and harvest age. Variations in the harvesting age of a melon depend on several factors, namely cultivation methods, genetics, and the environment. In addition, the relatively long harvesting age is also due to the cultivation method used. The hydroponic cultivation system in the 1432 masl highlands can inhibit the generative development of plants because the dose of nutrients does not match the plant's needs. The level of sweetness, the weight of fruit, and the thickness of fruit flesh are caused by nutritional factors. Conventional methods in the soil allow plants to meet nutrient requirements independently.

Microelements that may not be present in hydroponic nutrition can significantly affect the quantitative character of the fruit. At the same time, the shelf life of fruit is related to the type of melon as a climacteric group which has a significant increase in respiration rate before fruit ripening. Another factor that affects the shelf life of fruit is ethylene content which can accelerate fruit ripening and spoilage. Melon fruit color is controlled by carotenoid and flavonoid pigments. The color combination commonly found is a combination of the activities of the two pigments encoded by the CBP (Carotenoid Biosynthesis Pathway) gene family (Stanley and Yuan 2019). Melon flesh color is controlled by the Golden SNP (single nucleotide polymorphism) of the Orange-gene (CmOr), which predominantly triggers the accumulation of pro-vitamin A and beta-carotene molecules in the fruit mesocarp (Chayut et al. 2021). The stability of the characterizing characters and the uniformity between individuals indicates that the Meloni cultivar's requirements as a new cultivar have been fulfilled.

Traditional identification approaches based on morphological characters are considered less effective, uninformative, and time-consuming. Phenotypic traits are highly plastic, often mutagenic, depending on environmental conditions, and are not available at all stages of growth (Korir et al. 2012). Therefore, assessments based on morphological characters tend to be non-objective and are much influenced by the observer's subjectivity. Recent molecular markers have been widely developed for species authentication, such as phylogenetic observations, allele frequency analysis, DNA identification, and genebank management (Hyun et al. 2020). Because this approach is based on DNA characters, it can be found in all organs and plant life cycles. Molecular markers commonly used in cultivar identification are RFLP, RAPD, SSR, ISSRs, AFLP, SNPs, SAMPL, M-AFLP, SRAP, CAPS, SCoT, DNA sequencing, and DNA micro-arrays (Korir et al. 2012)

In this study, the identification of molecular characters using molecular markers ISSRs. The molecular markers of ISSRs have a higher sensitivity to fragment polymorphisms than other dominant molecular markers (Almeida-Pereira et al. 2017). ISSR can detect the level of polymorphism of an individual without knowing its DNA sequence, so it is widely used for characterizing genetic relationships between populations (Abdalla et al. 2020), and amplifying sequences between microsatellites can quickly distinguish closely related individuals (Buhroo et al. 2018). Determination of kinship between cultivars using a phenetic approach based on the similarity of morphological and molecular characters. These characters are used as OTU (Operational Taxonomic Unit), analyzed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) grouping method, and the Jaccard coefficient descriptive method.

Genetic distance is the degree of difference in genes in a genome between species or between populations within a species as measured by numerical methods (Dogan and Dogan 2016). Meanwhile, the similarity index shows the similarity of phenotypic characters and relatively close phenetic relationships. The identity of Meloni as a new cultivar has also been validated previously using close determinants such as Melonia and Melona. Based on the Jaccard coefficient, the similarity index between Meloni and Melonia is 85.5%, while between Meloni and Melona is 73.1%. The high similarity between Meloni and Melona is high because Melonia is a variety resulting from a cross between Meloni and Melona (Daryono et al. 2019).

Table 7. Percentage of DNA polymorphism of Meloni cultivar at different cultivation sites

ISSRs primer	Total amplified DNA	Polymorphic loci	Polymorphic rate (%)	Fragment size (bp)
UBC 807	4	0	0	354-592
UBC 808	4	0	0	647-1200
UBC 810	3	0	0	685-862
UBC 812	5	3	60	419-1303
UBC 825	6	4	67	544-1387
Average	5	1.4	25.3	-
Total	22	7	-	-

Table 8. Similarity matrix on individual Meloni cultivars based on UPGMA with Jaccard coefficient

Individuals	Bandung, West Java			Sleman, Yogyakarta		
	MLI.1.N	MLI.2.N	MLI.3.N	MLI.1.M	MLI.2.M	MLI.3.M
MLI.1.N	1.00					
MLI.2.N	1.00	1.00				
MLI.3.N	1.00	1.00	1.00			
MLI.1.M	0.74	0.74	0.74	1.00		
MLI.2.M	0.76	0.76	0.76	0.88	1.00	
MLI.3.M	0.78	0.78	0.78	0.90	0.93	1.00

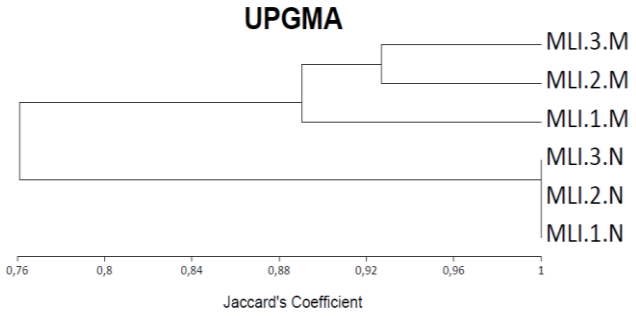

Figure 3. Phenetic relationship on individual Meloni cultivars based on UPGMA results with Jaccard coefficient approach. MLI: Meloni cultivar. Cultivation sites; M: Sleman Yogyakarta, N: Bandung, West Java, Indonesia

The conclusion obtained based on the previous description is that the traditional approach to identifying a species based on morphological characters remains essential and provides an accurate picture of the distinctive character of a cultivar. On the other hand, identification is based on molecular markers because they can validate intra-species genetic variation. The results of DNA identification provide comprehensive information on the specificity, stability, and genotype uniformity of a cultivar that is fast, accurate, and stable. Therefore, characterization of phenotype and genotype on Meloni cultivar provides objective evidence with accurate and comprehensive data to prove the requirements as a new cultivar. The main result of this research is the acquisition of Plant Variety Protection Rights (PVT) for the Meloni cultivar with No. 00486/PPVT/S/2020 by the Ministry of Agriculture of the Republic of Indonesia.

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