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Assessment of maturity stage and stability of new Indonesian melon cultivar 'Melona' based on ISSR markers and morphological characteristics

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Abstract. *Yusuf AF, Alfiani A, Salsabila TAS, Kusnanda PS, Hidayati IN, Daryono BS. 2023. Assessment of maturity stage and stability of new Indonesian melon cultivar 'Melona' based on ISSR markers and morphological characteristics. Biodiversitas 24: 308-316.* Market preferences for horticultural products determine the product's selling value and absorption. One market criterion often used for the quality of horticultural products is color and maturity level. Color differences can provide a representation of the metabolites contained in a product. This study provides a standard for assessing melons' maturity level, which will be helpful as a benchmark for Indonesian consumer acceptance. In addition, this study also provides information on the stability of the character of the new cultivar 'Melona' from several generations of crosses to obtain superior cultivars. Assessment of maturity level based on color development in 'Melona' is divided into five stages. In the first and second stage, fruit was dominated by green color with Increasing fruit volume. The color change begins in the third stage. in the third stage and the following phases, there is an increase in the accumulation of beta-carotene and characterized by the dominance of orange instead of green. Maximum ripe of 'Melona' is characterized by strong yellow lobes, creamy-white interlobes, and orange flesh color. A molecular analysis employing four ISSR primers (UBC-807, UBC-808, UBC-811, and UBC-824) resulted in high uniformity and low genetic variation in the hybrid and parent lines genotype. Analysis resulted a total of 42 DNA fragments with only one polymorphic DNA fragment. The polymorphic DNA fragment was 2,300 bp in size and was found in the female accession 'Melona' and the hybrid 'Melona' based on the UBC-808 primer. The numerical phenetic relationship between male, female and hybrid accession melon cultivars is very close (above 97.6%) and indicated that the melona cultivars were stable.

Keywords: *Cucumis melo* 'Melona', ISSR, maturity stage assessment, phenotypic

INTRODUCTION

Market preferences for horticultural products determine the product's selling value and product absorption. The fruit color is one of the factors that influence consumers' attractiveness. Fruit color preference is based on attractive taste and representation of metabolite content. Different color variations in fruit also affect all organoleptic and metabolite compounds. The color indicates fruit ripening which involves several physiological processes, from the breakdown of chlorophyll to the accumulation of β-carotene and other compounds. For example, in eggplant cultivars, color variation is influenced by delphinidin-3-rutinoside (D3R) and delphinidin-3-(p-coumaroyl rutinoside)-5-glucoside or nasunin. The different molecules give variations in the peel color from black to purple. The color of deep black eggplants represents the concentration of nasunin in cultivars from Japan (Florio et al. 2015), which is the underlying consumer acceptance of eggplant products.

'Melona' is a cultivar developed by the Genetics and Breeding Laboratory, Gadjah Mada University (UGM), Yogyakarta, Indonesia. Fruit is rich in β-carotene antioxidants because it has an orange fruit character. Orange flesh melon has low chlorophyll but is high in beta-carotene content (Chayut et al. 2021). Therefore, standards for assessing melons' maturity level at several ripening stages are required. The maturity level assessment is also related to consumer preferences and the demand for the highest levels of beta-carotene. In addition, the 'Melona' cultivar is a new cultivar that still requires protection in Plant Variety Protection (PVP). Therefore, it is necessary to support molecular data to validate the morphological characteristics of the fruit on the 'Melona' cultivar.

The crossing is one of the efforts in traditional plant breeding to obtain superior plant cultivars. From the results, hybrid cultivars are expected to have superior characteristics from both parents. Unfortunately, this traditional method has limited objectives that can be expanded. New plant breeding techniques (NPBTs) are the latest techniques developed to overcome the limits of plant breeding in tree species. This method has been applied in citrus breeding because of its complex species biology, such as the absence of seeds, apomixis, high heterozygosity, and prolonged juvenile phase (Salonia et al. 2020). The breeding of melon 'Melona' is, to date, still employing the traditional breeding methods because the species' biology is relatively simple.

One of the problems in horticultural cultivation is seed quality. A further understanding was conveyed that self-produced seeds in plants have the potential to produce more optimal yields. This phenomenon can occur due to the suitability of soil conditions but requires breeding for ten generations of planting (Katsu et al. 2021). Advances in molecular biology are accelerating the development of targeted superior cultivars (Salgotra and Stewart 2020). The NGS method (next-generation sequencing) is a different method to genome-wide DNA polymorphism analysis in Chinese Cabbage Plants (Kim et al. 2021). New cultivars must have a stable, uniform, and different character from other cultivars. The stability and uniformity of a cultivar are obtained with low genetic variation and heterozygosity.

ISSR (Inter-Simple Sequence Repeat) is one of the molecular markers used to study genetic variation. ISSR is a part of the genome between microsatellite sequences in the opposite direction. ISSR is more accurate than RAPD and AFLP because it is stable, effective, efficient (Mohammadabadi et al. 2021), and can distinguish genotypes in the population (Daryono et al. 2019). This study aimed to determine a maturity stage and predict morphological characters in the melon cultivar 'Melona' as a description of consumer preferences and reveal the molecular character of ISSR to provide information on the stability and uniformity of the Melona cultivar.

MATERIALS AND METHODS

Study area

Cultivation and assembly of hybrid 'Melona' were carried out in Jamusan greenhouse, Bokoharjo, Prambanan, Sleman, Daerah Istimewa Yogyakarta, Indonesia. In addition, morphological data collection, maturity level assessment, and molecular analysis were carried out at the Genetics and Breeding Laboratory, Faculty of Biology, Gadjah Mada University (UGM), Yogyakarta, Indonesia.

Procedures

Hybrid melon assembly

Cultivars were assembled by crossing male and hermaphrodite flowers from plants with different specificities. The specificity refers to the determination of male and female individuals according to fruit, seeds, and flowering characteristics. For example, the female parent 'Melona' was chosen for its large and high number of seeds produced. Meanwhile, the male parent ‘Sires’ was selected based on its superior qualitative characteristics and the high number of male flowers it produced (Daryono and Maryanto 2017). Crosses were carried out in the morning from 06.00-07.00 am by manual castration and pollination.

Phenotypic characterization and maturity stages assessment

The observed characters included qualitative and quantitative characters. The observed characters referred to the PERMENTAN Number 01/Pert/SR.120/2/2006 Article 14 and Guidelines for Compiling Descriptions and Testing the Truth of Horticultural Cultivars issued by the Ministry of Agriculture. The determination of the level of maturity was based on differences in the color of the fruit skin (lobes and interlobes) and the color of the fruit flesh. Color discrimination was carried out using an RHS color chart, and the quantitative character identification was done using a ruler. Data for the stability analysis were obtained from the plant populations evaluated for three generations (G2, G3, and G4); 10 individuals (10% of population) for each generation were selected to identify morphological characters.

Sample collection and DNA extraction

Molecular analysis was carried out with four replications. The samples used in this study were the leaves of the male, female, and hybrid-specific 'Melona' cultivars. Sampling was conducted when the plant was 3-4 weeks old from young leaves (3rd to 5th leaf from the tip), free of fungal and viral infections. The leaves were put in ziplock plastic, coded, and stored in the freezer at -20°C. DNA extraction was done using the Nucleon-Phytopure Kit Illustra DNA Extraction Kit PhytopureTM RPN 8511 consisting of Reagent 1, Reagent 2, and Resin. The implementation procedure followed the protocol provided in the kit. The extracted DNA was then qualitatively measured for concentration and purity using a spectrophotometer (Nanovue Plus).

DNA amplification by PCR-ISSR

DNA amplification was performed using a DNA PCR Kit (2x My Taq HS Red Mix Bioline) and ISSR primers; UBC 807; UBC 808; UBC 811, and UBC 824 (Table 1) (Innark et al. 2014) with modification. PCR Kit Reagents consisted of 12.5 µL of Bioline, 8.5 µL of sterile ddH2O, 2 µL of ISSR primer, and 2 µL of template DNA were inserted into a microtube and homogenized with a vortex. The amplification followed the melon genome amplification procedure previously carried out using the BOECO Thermal cycler PCR. The amplification results were analyzed qualitatively using 1.8% agarose gel by electrophoresis. In addition, the amplicons were visualized using a combination of GelDoc and UV transilluminator as documentation media. First, the DNA fragments formed appeared to glow white, and then the visible DNA fragments were observed.

Table 1. ISSR primers with nucleotide sequences and annealing temperatures

Primer	Primer sequence 5’-3’	Annealing temp. (°C)
UBC-807	AGAGAGAGAGAGAGAGT	43.8
UBC-808	AGAGAGAGAGAGAGAGC	43.8
UBC-811	GAGAGAGAGAGAGAGAC	44.8
UBC-824	TCTCTCTCTCTCTCTCG	40

Data analysis

Genetic variations among the parents and hybrid lines of 'Melona' were analyzed based on DNA profiles (electrophoretic DNA fragments). A cluster analysis based on Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) and the Jaccard coefficient was conducted using MVSP software, while the morphological analysis and fruit maturity assessment were processed using Corel Draw 2021 and Microsoft Office 365. The quantitative character was subjected to a one-way ANOVA, followed by a post hoc test at a 0.05 significant level. The ANOVA was performed using IBM SPSS.

RESULTS AND DISCUSSION

Maturity stage assessment of 'Melona' fruit

The fruit development of the 'Melona' cultivar was divided into 5 phases based on peel fruit (Figure 1) and mesocarp color (Figure 2). Color is determined between the color of the object and the standard color on the Royal Horticultural Society Color Charts. This phase refers to the color changes that occur in the 'Melona' fruit.

Based on Figure 1, it is known that the skin color of the Melona fruit is interesting. Unlike other melon cultivars, the 'Melona' cultivar has a specific shape: the presence of lobes and interlobes. This causes color variations between the interlobes and lobes, which can indicate ripeness in the 'Melona' cultivar. Before stage 1 (14 DAA), there were several pre-stages of the development phase resulting from the pollination process with a dark green color complex (RHS 143A) in both lobes and interlobes (data not shown). The first phase is marked with a dark green color on the interlobes and lobes (144A). Interlobes in this phase are still indentations and have not yet formed distinct color complexes. In the second phase, the fruit undergoes enlargement and a change in the primary color complex (lobes) from dark green to light green (RHS 144C) in the lobes. Meanwhile, the interlobes are clear with a darker color complex than the lobes (145 A). This green color complex is due to the dominance of chlorophyll pigment. Finally, in the third phase, the fruit undergoes significant color changes. Peel fruit began to be dominated by yellow (RHS 151C). This color complex is probably caused by the initial change of chlorophyll to carotenoids. We refer to this phase and the hereafter phase as the fruit ripening phase because the increase in size does not occur significantly with complex color changes. Subsequent development (phase 4) is marked by a strong yellow color complex on the fruit skin (RHS 7A). The difference from the previous phase is the fading of the interlobes color to become whiter (RHS 145D). In the last phase (fifth phase), the fruit was maximally ripe and marked with white interlobes (RHS 145D) and strong yellow color (RHS 12 A) on the peeled fruit. Sometimes the fruit has separated from the stalk at this stage and can be used for the future breeding program.

The ripening phase of the fruit was also marked by an increased accumulation of carotenoid pigments in the fruit flesh, which formed an orange complex (Figure 2). Based on Figure 2, fruit ripening was indicated by the increasing intensity of the orange color on the flesh fruit. Green complexes (RHS 145D and RHS 145C) were found in the first (14 DAA) and the second (21 DAA) stages. The fruit's flesh at both sets is still green, indicating the dominant chlorophyll content. The change in the color of the 'Melona' flesh begins in the third phase (28 DAA). In this phase, the development of the orange color complex has begun to appear from the innermost area (fruit cavity compartment) to the outer area. It forms a green-orange gradation character (Figure 2). The dominance of orange color was found in the fourth phase (35 DAA) with an orange color complex (RHS 25C). In this phase, the fruit's skin also tended to be green, indicating that the fruit was not fully ripe. Finally, in the last phase, the intensity of the orange color in the fruit flesh increases (RHS 24C). The fruit skin was creamy-white and could be marked by the release of the fruit from the stalk.

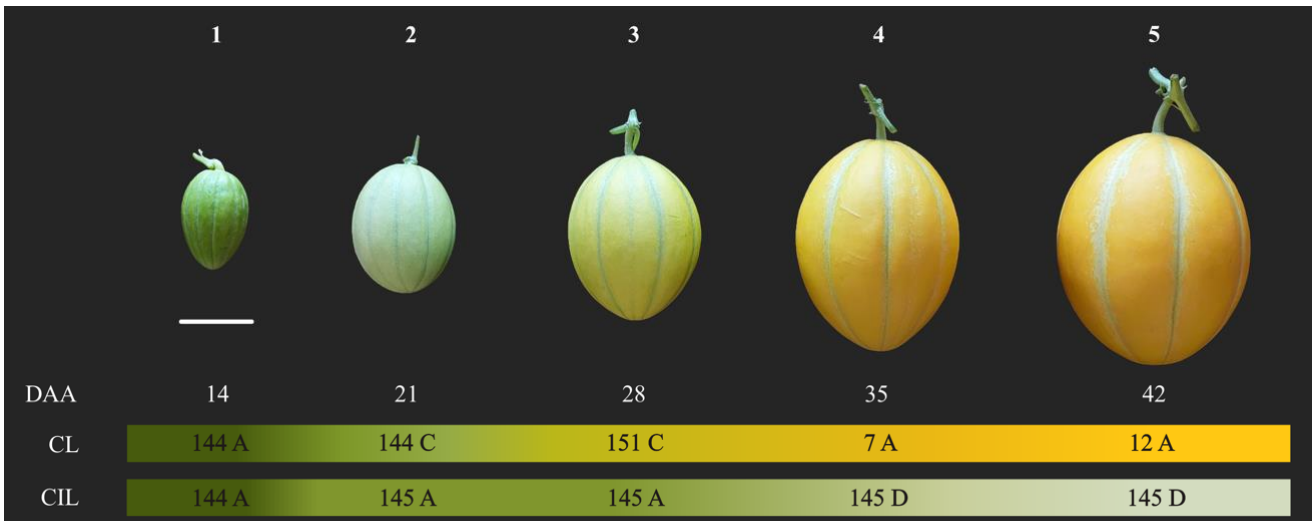

Figure 1. Maturity stage based on peel fruit color of 'Melona'. Note: DAA (days after anthesis); CL (color of the lobe); CIL (color of interlobe); The number (1, 2, 3, 4, 5) indicates five maturity stages; scale size 5 cm

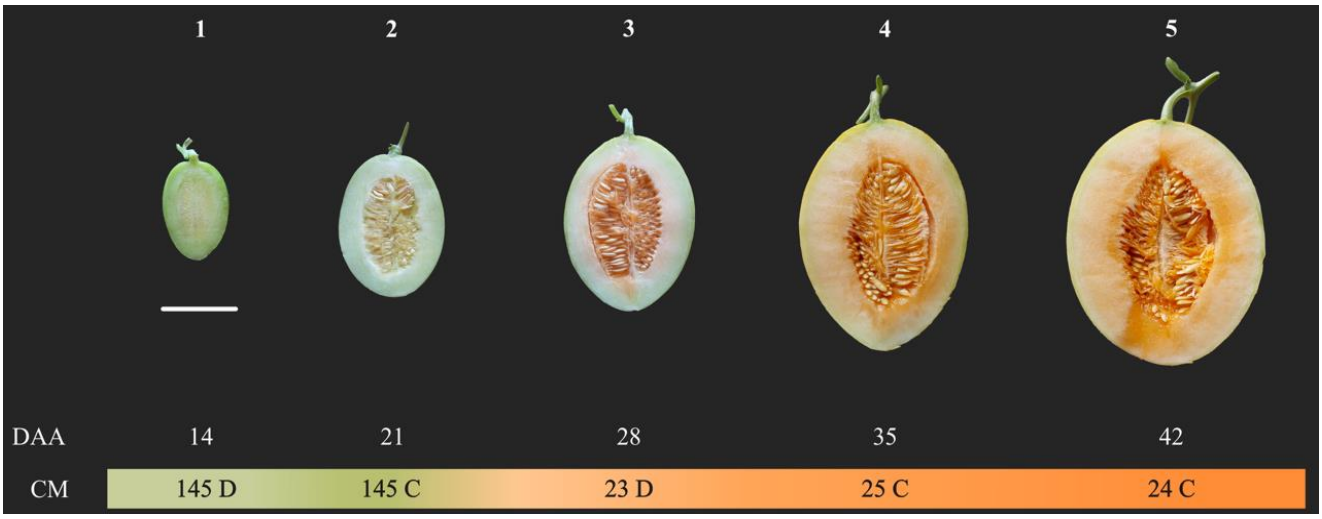

Figure 2. Maturity stage based on peel fruit color of 'Melona'. Note: DAA (day after anthesis); CM (color of mesocarp); The number (1, 2, 3, 4, 5) indicate five maturity stages; scale size 5 cm

Stability of 'Melona' cultivars between generation

Based on Table 1, an analysis of the morphological characters of the three generations provided a stability overview of the 'Melona' cultivar. The different characteristics of cross-generation were marked with an asterisk (*); there are stem color, rod diameter, leaf length, leaf width, fruit weight, the perimeter of fruit, vertical diameter, horizontal diameter, fruit skin thickness, mesocarp thickness, seed length, and a sweetness level (Brix). Phenotypic variation had a continuous distribution pattern, indicating a quantitative character. Many complex genes regulate quantitative phenotypic variation. These genes have a small effect, but their effects are sensitive to environmental changes. The overall quantitative characteristics of G_2 and G_4 were almost higher than G_3, except for the character of sweetness and thickness of the mesocarp based on ANOVA result with a significance level of 5%. The higher quantitative character is dominated by 'Melona' in the fourth generation. This is caused by G_4 being cultivated in conventional greenhouses with soil cultivation media on fresh soil media rich in nutrients. The sweetness level of G_3 was higher due to the abundant input of nutrients from the hydroponic method. Whereas in the G_3 and G_4 with conventional soil medium methods, nutrient modification cannot be carried out intensively.

Morphological differences between the parent and hybrid lines

Morphological characters were measured during the hybrid assembly process to differentiate them from other genotypes. Genotypes in this study refer to the parents and hybrid lines of 'Melona' cultivars. The selection of male and female parental characters was based on morphological characters, including qualitative and quantitative characters. The male parent (♂) was selected based on the number of male flowers and striking qualitative characters. In contrast, the female parent (♀) was selected based on the superiority of quantitative character and the number of seeds. Based on Table 2. The difference between the parents and hybrid lines of 'Melona' was found in the fruit weight, Brix or sweetness level, flesh-fruit weight, the weight of cavity compartment, vertical and horizontal circumference, base fruit diameter, vertical diameter, horizontal diameter, skin-fruit thickness, flesh-fruit thickness, and the number of seeds. The difference is indicated by an asterisk (*) in Table 2. These different characters were only found in quantitative characters, which were analyzed using one-way ANOVA at a significance level of 5%. Hybrid lines of 'Melona' provide a superior characteristic to the parent lines.

Molecular characteristics of 'Melona' based on ISSRs marker

The PCR amplification using the UBC-807 primer produced ten amplified monomorphic DNA fragments with a size of 226-1455 bp. UBC-808 primer yielded a total of 13 DNA fragments with one polymorphic DNA fragment (Figure 3). The size of all these fragments ranged from 180-2300 bp. This primer showed the presence of a specific DNA fragment measuring 2,300 bp in the female parent and hybrid lines 'Melona'. UBC-811 primer produced 13 DNA fragments from 218-2.127 bp. DNA amplification using UBC-824 primer produced 6 DNA fragments with sizes ranging from 643-3,720 bp. Like the amplification results in primers 807 and 811, the UBC-824 primer only produced monomorphic DNA fragments indicating low genetic variation in the 'Melona' cultivars.

The PCR amplification using 4 ISSR primers resulted in 41 monomorphic DNA fragments and one polymorphic fragment. The findings are because the ISSR primer amplifies the region between the microsatellites. The number of fragments produced in the amplification process is influenced by the number of parts of genomic DNA complemented by the sequence of primary nucleotide

bases. From the electropherogram scoring results for each primer, the percentage of polymorphism can be determined by comparing the polymorphic DNA fragments with the total DNA bands formed.

Phenetic relationship of the parents and hybrid lines of 'Melona' cultivars

Phenetic relationships based on character similarities can illustrate the closeness of the relationship between hybrid lines and their parents. For example, the percentage of relationship between female 'Melona' and hybrids is 100%, while the male 'Melona' relationship with female 'Melona' and hybrids is 97.6%. This shows that the hybrid line 'Melona' has a dominant character and tends to be like the female parental line 'Melona'. This is in accordance with the purpose of breeding where several female lines characters such as large size and abundant female flowers are obtained. Without neglecting the character of the male lines, which are still inherited in the hybrid lines 'Melona' (Figure 4).

Discussion

'Melona' results from applying selective breeding to a unique phenomenon in commercial cultivars. This phenomenon is possible due to mutation or atavism. Mutation is a change in the DNA sequence of an organism's genome, while atavism is the reappearance of characters that previously did not appear in several generations. Unfortunately, until now, there has been no further research on the clarity of these two things in the 'Melona' genome.

The selection process is essential in developing hybrid 'Melona' cultivars. In general, the selection was performed to form pure lines with high homozygosity through selfing in 5-6 generations. Generations with high homozygotes can be used as conservation parents to develop further hybrid lines. But in the 'Melona' cultivar, the high homozygosity was obtained in 3-4 generations (Table 2). Especially in the orange peel fruit pattern with vertical lobes around the fruit (Figure 1). That is the identity character of 'Melona' to other melon cultivars.

Table 2. Morphological characteristics of the 'Melona' cultivar in the different generations

Morphological parameters	Generation		
	G_2	G_3	G_4
Plant type	Annual	Annual	Annual
Habitus	Herbaceous	Herbaceous	Herbaceous
Leaf shape	Triangularis	Triangularis	Triangularis
Rod shape	Cylindrical	Cylindrical	Cylindrical
Flower shape	Rotate	Rotate	Rotate
Fruit shape	Ovate	Ovate	Ovate
Seed shape	Tapered ellipse	Tapered ellipse	Tapered ellipse
Fruity scent	Moderate	Moderate	Moderate
Fruit skin type	Lobed	Lobed	Lobed
Flowering type	Andromonoecious	Andromonoecious	Andromonoecious
Rod color *	146D Moderate Yellow Green	146D Moderate Yellow Green	146C Moderate Yellow Green
Leaf color	137B Moderate Olive Green	137B Moderate Olive Green	137B Moderate Olive Green
Flower crown color	7A Brilliant Yellow	7A Brilliant Yellow	7A Brilliant Yellow
Flower crown color	7A Brilliant Yellow	7A Brilliant Yellow	7A Brilliant Yellow
Flower petal color	144A Strong Yellow Green	144A Strong Yellow Green	144A Strong Yellow Green
Flower petal color	144A Strong Yellow Green	144A Strong Yellow Green	144A Strong Yellow Green
pistil color	N144B Strong Yellow	N144B Strong Yellow	N144B Strong Yellow
Stamen color	9B Vivid Yellow	9B Vivid Yellow	9B Vivid Yellow
Lobe color	14A Vivid Yellow	14A Vivid Yellow	14A Vivid Yellow
Lobe line color	10D Pale Greenish Yellow	10D Pale Greenish Yellow	10D Pale Greenish Yellow
Flesh color	24C Light Orange Yellow	24C Light Orange Yellow	24C Light Orange Yellow
Seed color	18D Pale Yellow	18D Pale Yellow	18D Pale Yellow
Rod diameter (cm)*	1.14 ± 0.04^{a}	0.95 ± 0.02^{b}	1.37 ± 0.03^{c}
Leaf length (cm)*	13.90 ± 0.18^{a}	15.81 ± 0.04^{b}	21.57 ± 0.23^{c}
Leaf width (cm)*	15.50 ± 0.37^{a}	18.13 ± 0.08^{b}	26.55 ± 0.16^{c}
Fruit weight (kg)*	1.06 ± 0.03^{a}	0.44 ± 0.01^{b}	1.05 ± 0.02^{a}
The perimeter of fruit (cm)*	38.32 ± 1.29^{a}	28.96 ± 0.22^{b}	37.89 ± 0.35^{a}
Vertical diameter (cm)*	16.79 ± 0.24^{a}	11.88 ± 0.12^{b}	15.94 ± 0.12^{c}
Horizontal diameter (cm)*	11.68 ± 0.12^{a}	9.46 ± 0.05^{b}	12.20 ± 0.15^{c}
Fruit skin thickness (cm)*	0.48 ± 0.03^{a}	0.32 ± 0.01^{b}	0.41 ± 0.03^{c}
Mesocarp thickness (cm)*	2.63 ± 0.09^{a}	3.24 ± 0.03^{b}	2.58 ± 0.09^{a}
Seed length (cm)*	1.52 ± 0.03^{a}	1.26 ± 0.02^{b}	1.69 ± 0.04^{c}
Seed width (cm)	0.49 ± 0.01^{a}	0.49 ± 0.01^{a}	0.50 ± 0.00^{a}
Sweetness level (^{o}brix)*	12.20 ± 0.34^{a}	15.3 ± 0.34^{b}	13.60 ± 0.22^{c}

Note: *different characters; lowercase letters denote comparison among generations on the observed characteristics at a significance level of 5%; (±) standard error value

Table 3. Morphological characteristics of 'Melona' cultivar among parental and hybrid lines

Morphological parameters	Genotype lines		
	'Melona' ♂	**'Melona' ♀**	**'Melona' Hybrid**
Plant type	Annual	Annual	Annual
Habitus	Herbaceous	Herbaceous	Herbaceous
Leaf-blade shape	Triangularis	Triangularis	Triangularis
Stem shape	Silindris	Silindris	Silindris
Flower shape	Rotate	Rotate	Rotate
Fruit shape	Ovate	Ovate	Ovate
Seed shape	Tapered ellipse	Tapered ellipse	Tapered ellipse
Fruit aroma	Moderate	Moderate	Moderate
Skin fruit type	Lobed	Lobed	Lobed
Flowering type	Andromonoecious	Andromonoecious	Andromonoecious
Fruit base color	RHS 12A	RHS 12A	RHS 12A
Lobe color	RHS 145D	RHS 145D	RHS 145D
Flesh color	RHS 23C	RHS 23C	RHS 23C
Fruit weight*	327 ± 25.43 [a]	495 ± 12.35 [b]	991 ± 13.61 [c]
Brix*	10.4 ± 0.43 [b]	8.4 ± 0.84 [a]	12.6 ± 0.97 [c]
Number of lobes	9.6 ± 0.27 [a]	9.7 ± 0.21 [a]	10 ± 0.00 [a]
Fruit flesh weight*	236.5 ± 20.41[a]	387 ± 13.75 [b]	213.5 ± 5.58 [a]
Fruit skin weight	78 ± 10.52 [a]	82 ± 4.16 [a]	79.5 ± 2.35 [a]
Fruit cavity compartment weight*	22.09 ± 2.70 [a]	34.28 ± 3.02 [b]	70.40 ± 0.61 [c]
Vertical circumference*	28.62 ± 0.99 [a]	33.44 ± 0.40 [b]	44.12 ± 1.22 [c]
Horizontal circumference*	26.76 ± 1.00 [a]	30.15 ± 0.30 [b]	36.72 ± 0.23 [c]
Diameter of the base fruit*	0.91 ± 0.07 [a]	1.04 ± 0.06 [a]	1.43 ± 0.03 [b]
Pistil remaining diameter	0.50 ± 0.09 [a]	0.44 ± 0.02 [a]	0.54 ± 0.04 [a]
Vertical diameter*	9.75 ± 0.38 [a]	11.86 ± 0.25 [b]	16.34 ± 0.19 [c]
Horizontal diameter*	8.35 ± 0.35 [a]	9.61 ± 0.08 [b]	11.6 ± 0.11 [c]
Fruit skin thickness*	0.31 ± 0.04 [a]	0.29 ± 0.03 [a]	0.46 ± 0.03 [b]
Flesh thickness*	1.93 ± 0.10 [a]	2.36 ± 0.14[b]	2.59 ± 0.07 [b]
Weight 100 seeds	3.27 ± 0.36 [a]	3.03 ± 0.29 [a]	3.57 ± 0.08 [a]
Number of seeds*	134.9 ± 30.7 [a]	186.9 ± 30.28 [a]	302.2 ± 24.74 [b]

Note: *different characters; lowercase letters denote comparison among generations on the observed characteristics at a significance level of 5%; (±) standard error value

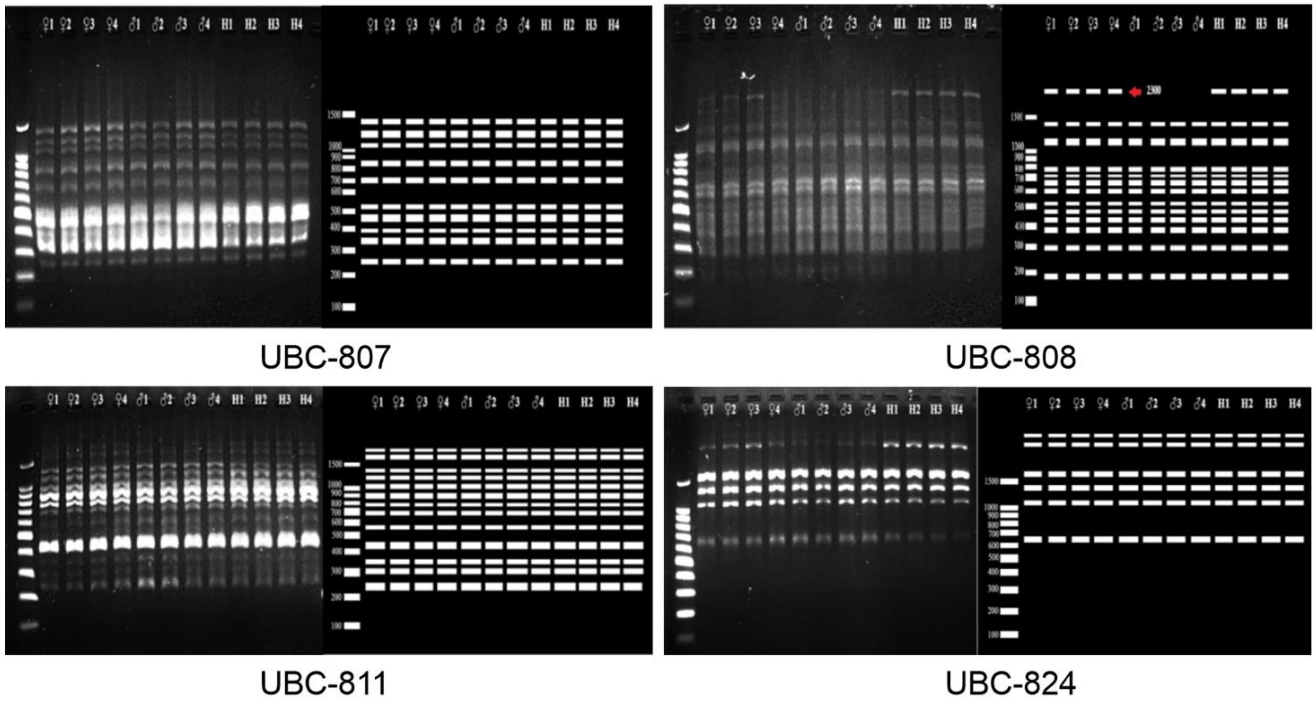

Figure 3. ISSR amplified bands (UBC-807, UBC-808, UBC-811, and UBC-824). Sample code; ♂ (female parent lines), ♀ (female parent lines), and H (hybrid lines). The number (1, 2, 3) indicate three replications of each genotype

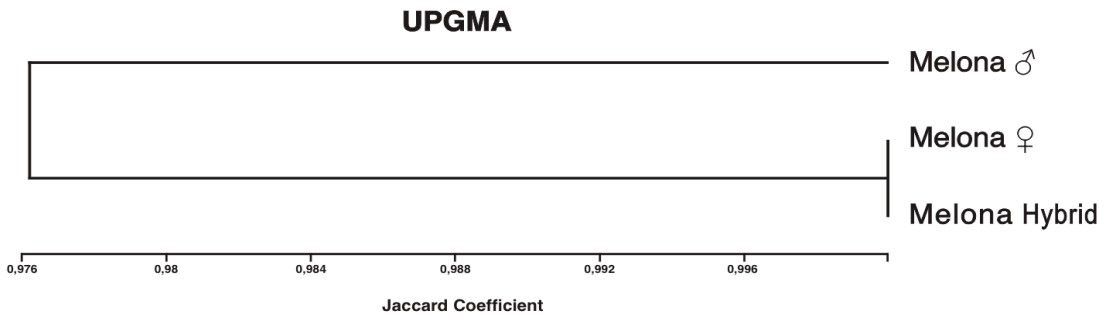

Figure 4. The phenetic relationship of the 'Melona' cultivar with the Jaccard Coefficient similarity index

Male and female lines were selected from pure lines. Female parent lines were selected based on dominant quantitative characteristics such as large fruit size. At the same time, male parent lines were determined based on superiority in qualitative characteristics such as taste, aroma, color, and the number of male flowers as pollen providers. In melon, sex determination is regulated by the inheritance of an *a* (andromonoecious) and *g* (gynoecious) alleles. Quantitative characters are controlled by polygenes, each of which has little influence on the appearance of the phenotype. Still, they complement each other to produce quantitative changes that can be observed (Daryono and Maryanto 2017). The environment influences the work of genotypes, so the expression of Qualitative Traits Locus (QTL), which is part of the genetic map that correlates to variations in quantitative traits, is also influenced by various environmental factors (Getahun et al. 2022).

Market acceptance of new cultivars requires definite data on when a cultivar can be harvested. In addition, the age of the plant determines the quality of the melons traded. Therefore, knowledge about fruit ripening in 'Melona' cultivars must be explored as a reference for harvesting these cultivars. Fruit ripening is a coordinated, complex developmental process that maximizes the organoleptic properties of the fruit. This coordination involves many genes that control several mechanisms of dramatic changes, such as changes in color, texture, firmness, taste, and aroma of the mesocarp in fleshy fruit, as well as sugar accumulation which is directly related to fruit quality (Osorio et al. 2013; Kumar et al. 2016; Shinozaki et al. 2018; Brumos 2021).

The fruit undergoes two stages, namely growth and fruit ripening. Hormonally, fruit growth is regulated by the hormones auxin (IAA) and cytokinin (CK). At the same time, the essential hormones that regulate fruit ripening and aging are ethylene (ET) and abscisic acid (ABA) (Zuo et al. 2020; Fenn and Giovannoni 2021). Based on the type and mechanism of ripening, fruit is divided into climacteric and non-climacteric. Climacteric fruit types are fruits that experience a spike in respiration and ethylene production after the fruit is harvested.

Meanwhile, the fruit that did not experience a spike was a non-climacteric fruit (Chen et al. 2018). The hormonal regulation of the two types of fruit is different. In climacteric fruits such as tomatoes, fruit ripening is regulated by the hormone ethylene. Meanwhile, non-climacteric fruit is regulated by the hormone ABA (Chen et al. 2020). ABA biosynthesis is initiated by breaking the β-carotenes 9'-cis-violaxanthin and 9'-cis-neoxanthin molecules into xanthoxins. This process is catalyzed by the NCED enzyme (9-cis-epoxycarotenoid dioxygenase) in plants which gradually increases the accumulation of ABA during non-climacteric fruit ripening (Li et al. 2022). In addition to biosynthesis, ABA regulation is also carried out through catabolism processes. The primary breakdown process occurs in the cytoplasm, catalyzed by the cytochrome P450 enzyme ABA 8'-hydroxylase to produce 8'-hydroxy ABA and phaseic acid. In addition, there is an alternative ABA catabolism mechanism through conjugation (Gupta et al. 2022).

The wide diversity of melon cultivars is an alternative model to determine the coexistence of climacteric and non-climacteric in one species. An example of a melon plant belonging to a climacteric is Védrantais cantalupensis type, while a non-climacteric of melon can be described by Piel de Sapo inodorus type (Pereira et al. 2020). The character of the 'Melona' cultivar indicates a non-climacteric fruit type that the ripening process must be carried out while the fruit is still on the plant. This was done because of the nature of 'Melona' fruit which did not experience a spike in ethylene after harvesting. Therefore, the harvesting process must be carried out when the fruit has shown maximum maturity (Figure 1), characterized by bright orange lobes and whitening interlobes (Stage 6). The fundamental difference of this type lies in the absence of a ripening phase but directly in the decay phase. As a result, non-climacteric melon fruit storage tends to be shorter. The concentration of decay-stimulating ethylene also affects the expression of related genes. Several genes in the carotenoid pathway, including *PSY1*, are induced by ethylene (Kumar et al. 2016).

'Melona' cultivars are characterized by orange flesh when ripe. The orange color of the fruit is due to the presence of carotenoids. Not only plays a role in color expression, but some examples of carotenoids such as -carotene, lutein, capsanthin, and zeaxanthin also benefit the body. Among these types of carotenoids, -carotene is the dominant type in expressing yellow, orange, and red colors in plants. The -polygenes complexly regulate carotene pigment. The β-carotene expression occurs in the presence

of CBP (Carotenoid Biosynthesis Pathway) gene expression. The various genes included in CBP are *PSY* (*phytoene synthase*), PDS (*phytoene desaturase*), *Z-ISO* (*15-cis-zeta-carotene isomerase*), ZDS (*zeta-carotene desaturase*), CRTISO (*carotene isomerase*), and β-LCY (*lycopene β-cyclase*) which produces β-carotene products (Stanley and Yuan 2019; Zhao et al. 2022). Changes and color determination of the mesocarpium in melons combine chlorophyll pigments and carotenoids, most of which are β-carotene types. The accumulation of β-carotene controls the variation and intensity of color.

As a new cultivar, validation data is vital to establish ownership of a cultivar resulting from breeding. Molecular markers have developed in such a way and have become an identification tool widely used as supporting data for morphological character testing. The selection process can be done more easily and quickly using molecular markers because they are constant and not influenced by environmental factors. In addition, molecular markers can identify genetic variation between organisms (Hasan et al. 2021).

Several researchers have also carried out polymorphism analysis using ISSR primers. Seventeen accessions of *C. melo* var flexuosus landraces were analyzed using 14 ISSR primers, yielding 63 bands with 27 polymorphic loci. The highest polymorphic percentage was obtained from primers ISSR-5 and ISSR-10. This value can be used as a reference for primary selection that can obtain multiple polymorphism sites (Akash et al. 2020). Molecular characterization of melon landraces in Iran used 23 ISSR primers. High polymorphism was found in ten ISSR primers, which resulted in a band average of 17.30 for each primer and an average polymorphism percentage of 50.29%. The primer that produced the highest polymorphism percentage was ISSR-3 (5'-$(GA)_8$T-3'), with a polymorphism percentage of 80.95% and 21 full bands (Maleki et al. 2018).

The dendrogram construction in Figure 3 proves that the melon phenotype character 'Melona' is stable despite different generations. There is a high similarity due to the sample from one cultivar. Polymorphic DNA fragments were only found in the UBC-808 primer with a size of 2,300 bp in the female and hybrid 'Melona'. The result shows the traits carried by the female 'Melona' in the DNA fragment, which has a length of ±2,300 bp that is passed on to hybrid offspring. The polymorphism at the fragment length of 2,300 bp could be due to genotypic characters not inherited in the male 'Melona' during selection and sowing. In molecular characterization using RAPD primers, some DNA fragments are inherited from 'Luna' to 'Melona' G2 but not inherited from 'Melona' G3. These fragments are like the 464 bp fragment from the OPA-07 primer and the 730 bp fragment from the OPAX 16 primer found in 'Luna' and 'Melona' G2. However, these two fragments were not found in 'Melona' G3 (Latifah 2016).

The ISSR primers UBC-807 and UBC-808 were also used as molecular markers in the stability analysis of the 'Melona' cultivar cultivated at different locations and elevations. In 'Melona' cultivar, DNA amplified using UBC-807 primer was four monomorphic DNA fragments measuring 354-592 bp, while UBC-808 produced four monomorphic DNA fragments measuring 647-1200 bp (Yusuf et al. 2022). The sensitivity of the two primers in analyzing genetic variation can be demonstrated by testing on different cultivars. Primers UBC-807 and UBC-808 produced a high percentage of polymorphism, namely 50% and 83%, respectively. This value is the result of analysis of genetic variation in the 'Hikapel' cultivar with other comparison cultivars such as 'Kirani,' 'Kinanti,' 'Sonya,' 'Moonlight,' and 'Aisha. 'The high genetic variation indicates that the 'Hikapel' cultivar is specific as a distinct cultivar (Yusuf and Daryono 2021).

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