International Journal on Advanced Science Engineering Information Technology

Vol.12 (2022) No. 4
ISSN: 2088-5334

Genetic Stability Analysis Based on *Inter-Simple Sequence Repeat* and β-Carotene Content Analysis in Melon (*Cucumis melo* L. 'GAMA Melon Parfum')

Wiko Arif Wibowo [a], M. Fikri Al Rasyid [a], Syifa Evilia Maharani [a], Budi Setiadi Daryono [a,*]

[a] *Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Sleman 55281, Yogyakarta, Indonesia*
Corresponding author: [*]bs_daryono@mail.ugm.ac.id

***Abstract*— The 'Gama Melon Parfum' (GMP) melons is a variety of plant breeding resulting from the Faculty of Biology's genetics and breeding laboratory, Universitas Gadjah Mada. GMP melons have a unique phenotypic character of a bitter taste and a strong aroma that has the potential to be used for cosmetics and medicine. Stability and genetic variation test are necessary to ensure the quality control of 'GMP' melons for industrial raw materials. The content of carotenoids is also important to reveal in utilizing metabolites compounds. Phenotypic character analysis was performed by comparing fruit grown conventionally and hydroponically grown on 'GMP' melons. The molecular observation method is genetic variation using the PCR-ISSR method with 5 primer ISSR and comparison with other varieties, namely 'Hikapel', 'Sky Rocket', and 'PI371795'. The data analysis used the UPGMA method, and genetic similarity was estimated using Jaccard Coefficient with MVSP 3.1 program. The method for observing β-carotene content is the UV-Vis spectrophotometric method. The results obtained showed that hydroponically grown 'GMP' melons had a relatively smaller size and faded fruit color. However, phenetically, 'GMP' melons grown both hydroponically and conventionally are in one cluster with a similarity level of 80.9%. Genetic analysis on 'GMP' melons and comparison melons showed a high level of polymorphism of 58.97%. While the results of β-carotene analysis on 'GMP' melons were 140,829 g/100 gr. It can be concluded that GMP melon has a stable genetic character. Cultivation methods and environmental factors cause changes that occur in GMP melons.**

***Keywords*— β-carotene; *Cucumis melo* L.; genetic stability; gama melon parfum; ISSR.**

Manuscript received 30 Jun. 2021; revised 24 Sep. 2021; accepted 10 Jan. 2022. Date of publication 31 Aug. 2022.

I. INTRODUCTION

Melon is one horticulture commodity with high economic value and large genetic diversity [1]. Melon plants spread to the Middle East and Asia, then became an important horticultural commodity in India, Egypt, Iran, and China [2]. There are several different fruit variations with each passing civilization and culture that cultivated it. So far, each type of melon characterized by the shape of the fruit, the color of the fruit skin, the color of the fruit flesh, and the aroma and taste according to the cultivar of the melon [3].

The Gama Melon Perfume (GMP) cultivar is one of the melon cultivars developed by the Genetics and Breeding Laboratory, Faculty of Biology, Gadjah Mada University, from the results of crossing the melon broodstock ♀NO3 and ♂ MR5 [4]. GMP melons have a similar character to Duda'im melons, but there are unique characteristics in the form of a turbine structure in the fruit, bitter taste in the flesh, and the shape of the longitudinal pattern [5], [6]. Some of the volatile aromas of the melon have been identified and are dominated by acetate esters and non-acetate [7]. As a member of the Cucurbitaceae family of plants, GMP melons contain high levels of bitter-tasting nutrients known as cucurbitacin that possess immense pharmacological potential [8][9].

The utilization of GMP melons as industrial raw materials requires certainty of phenotypic stability and stability of metabolite content. GMP Phenotypes manifest a balance between the inherited tendency to remain the same (phenotypic stability) and the tendency to change in response to current environmental conditions (adaptation). Phenotype stability can be analyzed morphologically and molecularly. ISSR (Inter-Simple Sequence Repeat) markers are highly polymorphic and useful in genetic diversity studies. ISSR can detect variations between populations that are separated by location and variations in individuals within the population [10][11].

Melon contains diverse carotenoids responsible for the different colors of the fruit. Like in other fruits and vegetables, carotenoids are melon's main functional components and micronutrients. Investigation of carotenoid composition, content, and its metabolic pathway in melons should become an important field of melon breeding for quality estimation and nutrition breeding [12]. Carotenoid metabolism is highly dependent on fruit ripening and is related to the metabolism of volatile compounds [13].

The study aims to determine the genetic stability of cultivated 'GMP' melon hydroponically and conventionally at different areas and altitudes. The 'GMP' melon will also be analyzed for genetic variation and the level of similarity with the comparison melon. Furthermore, the analysis of β-carotene content can support fruit quality indicators used in the industrial raw material sector.

II. Material and Methods

This research was conducted from June 2020 – February 2021. GMP, Hikapel, and PI371795 melon seeds were obtained from the Genetics and Breeding Laboratory of the Faculty of Biology UGM, while Sky Rocket seeds were commercial seeds (PT. Known-You Seed). All samples were cultivated in Madurejo Village, Sleman district, D.I. Yogyakarta conventionally [14] at an altitude of 144 meters above sea level, while specifically GMP melons are also cultivated in Warnasari Village, Bandung district, West Java with a hydroponic system at an altitude of 1,400 meters above sea level. Samples of plant leaves were taken at the age of 30 DAP, while samples of GMP melons were taken during the harvesting process. The research consisted of 3 stages of the method: observing phenotypic characters, observing molecular characters, and testing β-carotene content.

A. Observation of phenotypic characters

Observation of phenotypic characters in GMP melons refers to the modified study previous study [3], [6]. Observation of phenotypic characters was only carried out on several key characters of GMP melons to compare conventional cultivation in Madurejo Village and hydroponic cultivation in Warnasari Village. The qualitative phenotypic characteristics observed were fruit shape, basic rind color, pattern rind color, aroma, taste, seed color, seed shape, turbine, and flesh color. While the quantitative phenotype characters include fruit weight, vertical circumference, horizontal circumference, turbine diameter, inner vertical diameter, inner horizontal diameter, sweetness level, and fruit flesh thickness.

B. DNA Extraction and amplification with PCR-ISSR

Each sample of 0.3 grams and mashed with a mortar. Extraction process was used Nucleon Phytopure GE Healthcare with modification by [14], which consists of reagent I, reagent II, phytopure resin, chloroform, and isopropyl alcohol 70%. The DNA obtained was dissolved in TE Buffer solution and stored at -20ºC. The amplification process uses the BIORAD T100TM Thermal Cycler PCR machine. The samples in the PCR machines were consisted of a mixture of 12.5 µL PCR kit Ready Mix (MyTaqTM HS Red Mix), 2 µL of DNA template (200 ng), 2 µL ISSR primer (Table I), and 8.5 µL distilled water with a total volume of 25 µL. The PCR reaction was carried out in 35 cycles, starting with the pre-denaturation at 95ºC for 3 minutes, the denaturation with 15 seconds at 95ºC, the annealing for 30 seconds at 40-50ºC, and extension for 45 seconds at 72ºC. After that, we continued to the final extension stage for 4 minutes at 72ºC. PCR amplification visualized on electrophoresis with 1.5% agarose gel stained with Florosafe DNA dye in 1x TBE buffer solution with 50 volts for 55 minutes. After that, it visualized with Geldoc UV Transilluminator

TABLE I
ISSR PRIMER DATA WITH THE SEQUENCES OF NUCLEOTIDES AND NITROGENOUS BASES

Primer	Sequence of Nucleotide (5' – 3')	Number of nitrogenous base
UBC-807	AGAGAGAGAGAGAGAGT	17
UBC-808	AGAGAGAGAGAGAGAGC	17
UBC-810	GAGAGAGAGAGAGAGAT	17
UBC-841	GAGAGAGAGAGAGAGATC	18
UBC-855	ACACACACACACACACYT	18

C. Analysis of β-carotene content

Three samples of 'GMP' melons were used with 3 replications for each sample. The analysis procedure for β-carotene levels consisted of fruit sample extraction, fractionation of β-carotene compounds, and determination of β-carotene levels by UV-Vis spectrophotometry.

Five grams of fruit samples used, mashed with a porcelain mortar, and add petroleum ether and acetone in a ratio of 1:1 for extraction. Then add anhydrous Na_2SO_4 and petroleum ether to a certain volume. Fractionation of β-carotene compounds was carried out by the column chromatography method [15]. All components in the column were activated at 180ºC for 2 hours. Washed the column with petroleum ether and acetone in a ratio of 10:1 to speed up the fractionation process and push the filtrate into the bottom. Collected the filtrate and diluted again with petroleum ether to a certain volume. After that, read the β-carotene levels at the absorbance wavelength of 450 nm.

D. Data Analysis

Converted the Molecular data derived from DNA fragments to matrix 0-1. Used the cluster analysis to show the dendogram construction of the phenetic relationship from the similarity matrix data using the UPGMA method. Genetic similarity estimated by using Jaccard's Coefficient with MVSP 3.1 program [14]. Biochemical analysis data consisting of β-carotene levels obtained from the results of sample analysis by spectrophotometry. The absorbance obtained from each sample recorded and converted into tabular form.

III. Results and Discussion

A. Phenotypic Comparison between GMP Melons in Yogyakarta and Bandung

Specifically, the phenotypic characters were compared based on the fruit organs of the GMP melons plant which were grown conventionally in Madurejo Village and hydroponically in Warnasari Village (Table II).

TABLE II
PHENOTYPIC CHARACTERS OF GMP MELONS

Characters	GMP Madurejo (Yogyakarta)	GMP Warnasari (Bandung)
Fruit shape	Oblate	Oblate
Basic rind color	RHS 2015 N163C Orange Group-Stronge Orange Yellow	RHS 2015 N163B Orange Group-Stronge Orange Yellow
Pattern rind color	RHS 2015 175A Orange Group-Moderate Reddish Brown	RHS 2015 172B Orange Group-Moderate Reddish Brown
Aroma	Very fragrant	Very fragrant
Taste	Bitter	Bitter
Seed color	RHS 2015 161C Greyed-Yellow Group-Pale Yellow	RHS 2015 161C Greyed-Yellow Group-Pale Yellow
Seed shape	Ellipse	Ellipse
Turbin	Presence	Presence
Flesh color	RHS 2015 158B Yellow White Group-Pale Yellow	RHS 2015 158B Yellow White Group-Pale Yellow
Fruit weight (g)	142 ± 22.53	104 ± 5.40
Vertical circumference (cm)	20.8 ± 1.25	18.02 ± 0.38
Horizontal circumference (cm)	20.98 ± 1.05	18.42 ± 0.68
Turbin diameter (cm)	5.1 ± 0.22	2.64 ± 0.86
Inner vertical diameter (cm)	6.34 ± 0.31	3.72 ± 0.30
Inner horizontal diameter (cm)	6.52 ± 0.38	3.86 ± 0.22
Kadar gula (Brix)	3 ± 0.00	4.2 ± 0.84
Fruit flesh thickness (cm)	1.28 ± 0.08	0.84 ± 0.09

The results obtained indicate that hydroponic cultivation of GMP melon in Warnasari village has a relatively smaller fruit size. The size of the fruit can be seen from the quantitative data presented in Table II, one of which is the weight of the GMP melon in Madurejo village which is 142 ± 22.53 grams, while the GMP melon cultivated in Warnasari village is only 104 ± 5.40 grams. In addition, the color expressed from melon plants in Warnasari village is more faded than melon plants in Yogyakarta (Fig.1).

Fig. 1 Comparison phenotypic characters of GMP melons fruit, (a) traditional cultivation; and (b) hydroponic cultivation. Red arrow: Turbin structure

Fruit morphology is one of the phenotypic characteristics that is expressed and visible so that it can be measured and differentiated qualitatively and quantitatively. Phenotypic characters expressed are influenced by genotype and environment [14], [16]. GMP melons grown hydroponically in Warnasari Village showed a smaller size, presumably due to limited root growth that affected nutrient absorption. The hydroponic system is a cultivation that utilizes limited land using special shelves and media [17]. While the root system in conventional cultivation in the soil causes root growth not to be limited by the media and can grow to get maximum nutrients for growth [18]. Although each planting method has optimal nutritional needs, conventional planting has the advantage of soil media which has more supporting factors [19]. In the soil, there is a lot of organic matter and soil microbiome that is able to symbiotically with plant roots so that plant growth is better [20].

Another factor related to the planting method is the difference in the height of the area. Madurejo village has an altitude of 144 meters above sea level (lowlands) which relatively has environmental factors that are more suitable for melon cultivation. Warnasari Village which has an altitude of 1400 meters above sea level (highlands) makes the growth of GMP melon plants a little hampered. Environmental factors that affect plant growth in this context are climatic factors, radiation (including light), cloudiness, precipitation, wind, air temperature, the humidity of the air, carbon dioxide content, and air pollution [21]. The highland area has the characteristics of lower temperature, high rainfall, and low sunlight intensity [22]. These factors are not in accordance with the characteristics of melon plants, which prefer temperatures in the range of 25-30oC, low-moderate rainfall, and moderate-high sunlight intensity [23]. Environmental factors in Warnasari Village as a highland area cause the phenotypes expressed in the fruit to be faded and smaller than Jamusan Village, which has lowland characteristics.

B. Visualization of DNA fragments

Visualization of DNA fragments obtained from 5 groups of melon samples (GMP cultivated in Bandung, GMP cultivated in Yogyakarta, Hikapel, Sky Rocket, and PI371795) using 5 ISSR primers (Table. I). The total number of DNA bands was 39 bands, consisting of 16 monomorphic DNA bands and 23 polymorphic DNA bands (58.97%) (Fig. 2).

Based on Fig. 2, primers UBC-807 and UBC-810 produced 4 bands (±200-475 bp) and 3 bands (±620-800 bp) polymorphic bands, respectively. Meanwhile, polymorphic bands were found in the UBC-808 primer (5 polymorphic bands from 9 bands), UBC-841 (8 polymorphic bands from 12 bands), and UBC-855 (10 polymorphic bands from 11 bands). The percentage of polymorphic band DNA obtained is presented in Table III.

Genetic variation describes the phenotypic diversity in nature. Genetic variation can be identified by looking at differences in the sequence of the bases [24]. Genetic variation can be determined by measuring genetic variation parameters; one of them is by looking the percentage value of polymorphic DNA. If the percentage of polymorphic DNA is ≥50%, it can be said that the sample tested has a low similarity index, so the result of genetic variation is high. Meanwhile, if the percentage of polymorphic DNA is ≤50%, it can be said that the sample tested has a high similarity index, so the result of genetic variation is low [25]. Primers that can be used to analyze genetic variations between 'GMP' melons and comparison melons with a polymorphic DNA percentage of ≥50% are UBC-808, UBC-841, and UBC-855 primers.

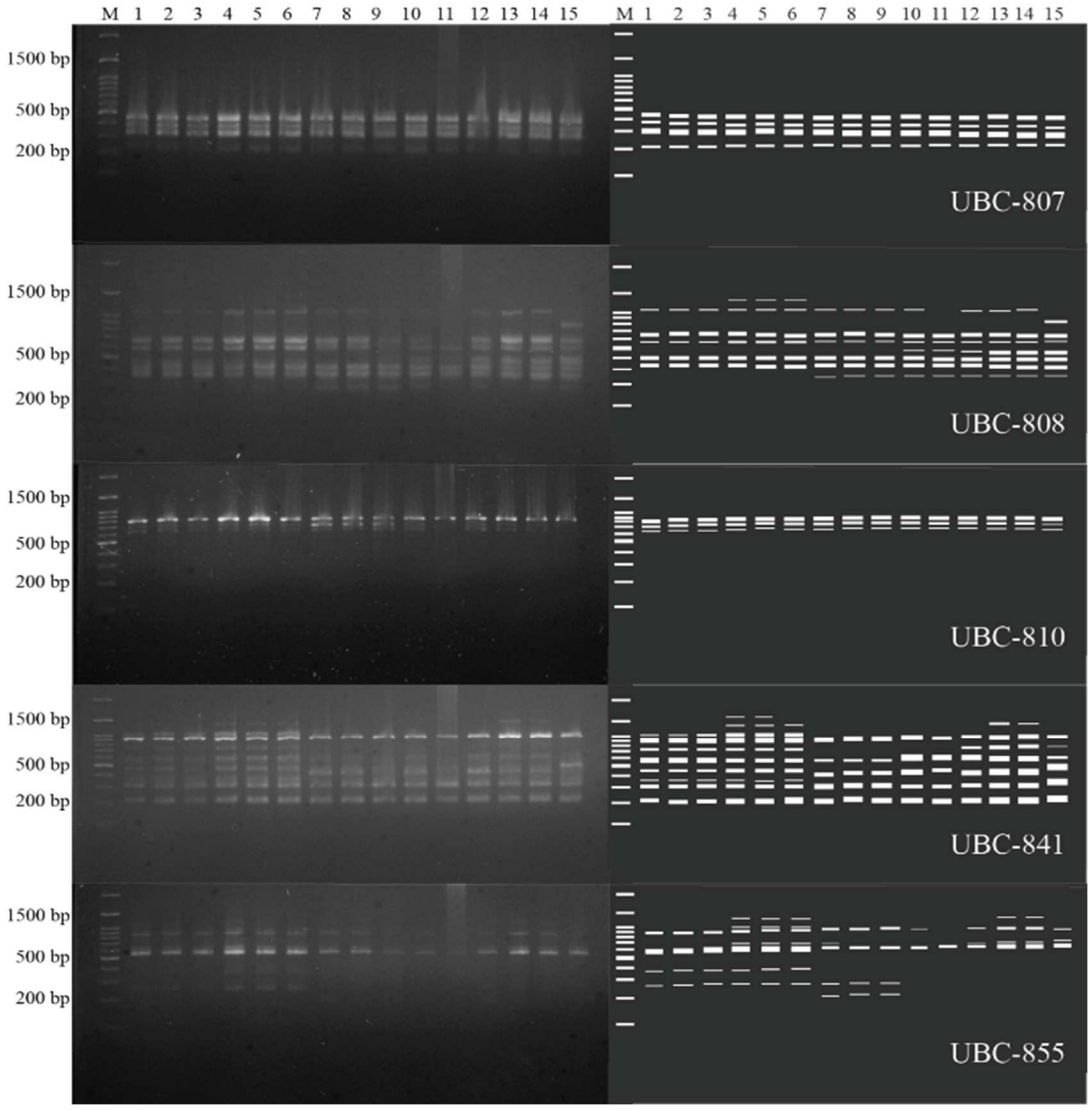

Fig. 2 The results of PCR visualization of fragments with fifteen DNA templates. The primers ID are shown in right side. (M: marker; 1-3: 'GMP' Madurejo; 4-6: 'GMP' Warnasari; 7-9: 'Hikapel', 10-12: 'Sky Rocket', 13-15: 'PI371795')

TABLE III
PERCENTAGE OF POLYMORPHIC DNA BANDS EACH ISSR PRIMER BETWEEN 'GMP' MELON SAMPLES AND COMPARISON MELONS

No	Primer	Number of DNA bands	Number of DNA monomorphic bands	Number of DNA polymorphic bands	Percentage of DNA polymorphic
1	UBC-807	4	4	0	0%
2	UBC-808	9	4	5	55,56%
3	UBC-810	3	3	0	0%
4	UBC-841	12	4	8	66,67 %
5	UBC-855	11	1	10	90,90%
Total		39	16	23	58,97%

Research by [14] analyzed the phenotypic and genetic stability of the new Indonesian melon cultivar (*Cucumis melo* L. 'Melonia') based on the ISSR molecular marker. This study used 4 ISSR primers including UBC-807, UBC-808, UBC-811, and UBC-824, resulting in a total percentage of polymorphic DNA bands of 31.7%. Genetic diversity can be seen from DNA polymorphism, so the application of ISSR molecular markers is very important because it can detect high polymorphisms, both in variations between geographically separated populations or individual variations in the population [26].

C. Phenetic Relationship between 'GMP' Melons and Comparison Melons

The dendogram of the similarity relationship (Fig. 3) divided into 2 big parts which separate the melon 'GMP' and the comparison melon ('Hikapel', 'Sky Rocket', 'PI371795'). The first part consisted of Madurejo 'GMP' melons and Warnasari 'GMP' melons. Meanwhile, the second part consists of the sample 'Hikapel', 'Sky Rocket', and 'PI371795'. 'GMP' melons planted with different cultivation systems and locations converged at a similarity level of 80.9%. 'PI371795', 'Hikapel', and 'Sky Rocket' converged at a similarity level of 77.1%. 'GMP' melons and comparison melons were converged at a similarity level of 59.1%.

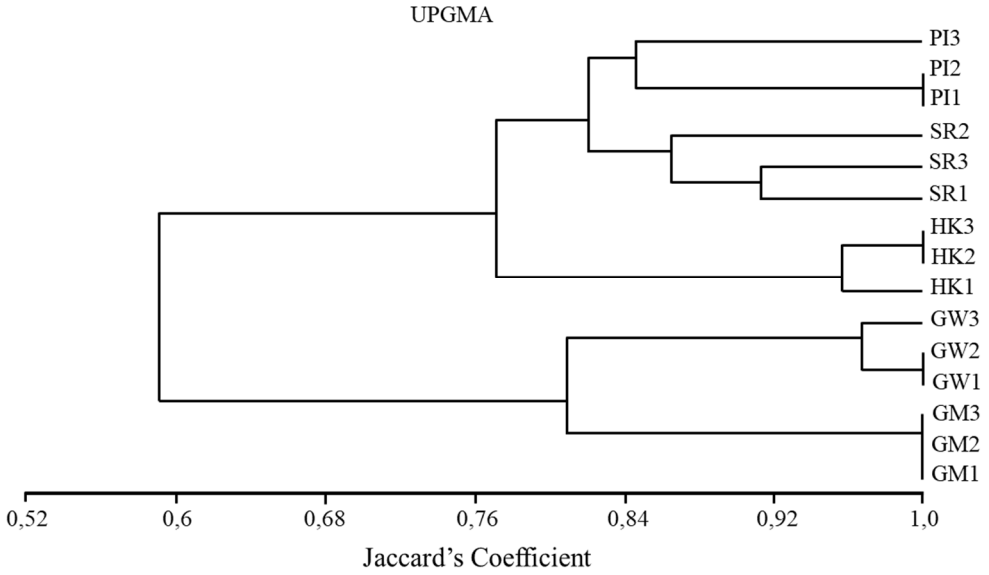

Fig. 3 The dendogram of the similarity relationship between 'GMP' melons and comparison melons based on Jaccard's Coefficient (GM: 'GMP' Madurejo; GW: 'GMP' Warnasari; HK: 'Hikapel'; SR: 'Sky Rocket'; PI: 'PI371795').

Cultivars grouping using numerical taxonomy is usually done using a phenetic approach [27]. The approach used to evaluate the phenetic similarity relationship can be in the form of morphological, anatomical, chemical, cytological, isozymic, or DNA characters [28]. Phenetic analysis is an approach that can be used to determine the relationship of a plant or animal based on similar morphological characters. The phenetic approach was structured based on all similarities to all existing characters, and the greater their similarities, the closer the relationship [29]. The striking difference between the analysis of phylogenetic and phenetic relationships is that the relationship in phenetics is based on the similarity in the properties of each group regardless of the history of their descent. In contrast, phylogenetic relationships are based on evolutionary assumptions as of the main reference.

The relationship can be determined using the Jaccard's Coefficient (Sj) method. The similarity index generated from the Jaccard's Coefficient method can be used as a reference in assessing the level of relationship of a cultivar. If the similarity index generated from the Sj method is ≥70%, it can be said that the sample has high similarity. If the similarity index is <70%, it can be said that the sample has low similarity [30]. The similarity index between Madurejo 'GMP' melons and Warnasari 'GMP' melons is ≥70%, indicating that 'GMP' melons grown in different cultivation systems and locations still have a high level of similarity. Meanwhile, the similarity index between 'GMP' melons and comparison melons is <70%. This is consistent with the hypothesis that the 'GMP' melon and the comparison melon have a low level of similarity. The 'GMP' melon is the result of crossing the broodstock of Natsu no Omoide ♀NO3 from Turkmenistan and Miyamauri ♂MR5, which originated from Japan [23]. The 'Sky Rocket' melon is a commercial melon mostly grown by Indonesian farmers. The 'Hikapel' melon is a melon developed by the Genetics Laboratory of the UGM Faculty of Biology. The 'Hikapel' melon itself is still related to the 'GMP' melon, where the 'Hikapel' melon results from a cross between the Hikadi melons and ♂ SL-3 melons. Meanwhile, 'Hikadi' melon itself results from crosses between melon broodstock ♀ GMP and ♂ La-3. Meanwhile, the 'PI371795' melon is a wild melon often used as broodstock in assembling melons from plant breeding [23].

D. Analysis of 'GMP' Melon β-Carotene Content

Analysis of β-carotene content aims to determine the content of β-carotene in 'GMP' melons. The sample of 'GMP' melon with the lowest levels of β-carotene was found in 'GMP' 2 melons with an average β-carotene level of 118.546 µg / 100g. Meanwhile, the sample of 'GMP' melon with the highest levels of β-carotene was found in 'GMP' 3 melons with an average level of β-carotene of 159.284 µg / 100g. The total average β-carotene content of the 3 'GMP' melons used was 140.829 µg / 100g (Table IV).

TABLE IV
RESULTS OF β-CAROTEN ANALYSIS

No	Code	β-carotene (µg/100g)			mean (µg/100g)
		1	2	3	
1	'GMP' 1	140,372	141,443	152,159	144,658
2	'GMP' 2	117,524	119,568	118,546	118,546
3	'GMP' 3	161,481	157,087	159,284	159,284
	Total				140,829

The β-carotene concentration (ug/g dry weight) in green flesh melon and orange flesh melon were 242.8 and 176.3, respectively[32]. GMP melons have different flesh colors, yellowish-white (RHS 2015 158B Yellow White Group-Pale Yellow). The difference in the color of the fruit flesh results in different β-carotene content. The levels of total carotenoid melon planted in Yogyakarta were higher at 706, 61 mg / 100 g than planted in Magetan, amounting to 292.16 mg / 100 g [33]. In addition, total carotenoids in fruit melon analyzed in the dry season is also higher than during the rainy season, which is 59.88 g / 100 for melons grown in Yogyakarta and

20.76 g / 100 g for melons grown in Magetan. This is because the levels of nutrients can be affected by several factors, including the planting location, season, time of harvest, variety, type, length of storage and other [16].

IV. Conclusions

There are differences in the phenotypic characters of GMP melons grown hydroponically in the highlands and those grown conventionally in the lowlands, but the molecular phenotypic characters still group GMP melons in the same cluster and separate from the comparison melons, namely Hikapel, Skyrocket, and PI371795. The β-carotene content of GMP melon is in the high range, so it has the potential to be used as industrial raw material.

Author Contribution

WAW supervised the research, data analysis, and wrote the manuscript, MFAR conducted the research and wrote the manuscript, SEM conducted the research and data analysis, and SS and BSD supervised and designed the research.

Acknowledgments

The authors are grateful to the Indonesian Ministry of Finance and the Indonesian Ministry of Research, Technology, and Higher Education for the funding support of this research through the RISPRO-LPDP. Also, this research is supported by Masters Education Towards Doctorate for Excellent Undergraduate scholarship program granted in 2020 with grant number: 3157/UN1.DITLIT/DIT-LIT/PT/2020

Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] R. Yano, S. Nonaka, and H. Ezura, "Melonet-DB, a Grand RNA-Seq Gene Expression Atlas in Melon (Cucumis melo L.)," *Plant Cell Physiol.*, vol. 59, no. 1, p. E4(1-15), 2018, doi: 10.1093/pcp/pcx193.

[2] A. Moing *et al.*, "Comparative metabolomics and molecular phylogenetics of melon (Cucumis melo, cucurbitaceae) biodiversity," *Metabolites*, vol. 10, no. 3, 2020, doi: 10.3390/metabo10030121.

[3] A. F. Yusuf, W. A. Wibowo, A. S. Subiastuti, and B. S. Daryono, "Morphological studies of stability and identity of melon (Cucumis melo L.) 'Hikapel' and comparative cultivars," in *AIP Conference Proceedings*, Sep. 2020, vol. 2260, pp. 030006(1–8), doi: 10.1063/5.0017606.

[4] W. A. Wibowo, M. I. Fatkhurohman, and B. S. Daryono, "Characterization and expression of cm-aat1 gene encoding alcohol acyl-transferase in melon fruit (Cucumis melo l.) 'hikapel,'" *Biodiversitas*, vol. 21, no. 7, 2020, doi: 10.13057/biodiv/d210722.

[5] H. S. Paris, Z. Amar, and E. Lev, "Medieval History of the Duda'im Melon (Cucumis melo, Cucurbitaceae)," *Econ. Bot.*, vol. 66, no. 3, pp. 276–284, 2012, doi: 10.1007/s12231-012-9205-4.

[6] W. A. Wibowo, S. D. Maryanto, and B. S. Daryono, "Phenotypic characters and identification CYPs (Cyclophilin) gene in Cucumis melo L. cv. Gama Melon Parfum," *Biodiversitas J. Biol. Divers.*, vol. 22, no. 6, pp. 3007–3014, May 2021, doi: 10.13057/biodiv/d220601.

[7] U. H. A. Hasbullah, Supriyadi, and B. S. Daryono, "Aroma volatile compounds profile of melon (Cucumis melo L.) cv. Gama Melon Parfum," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 292, pp. 012027(1–12), 2019, doi: 10.1088/1755-1315/292/1/012027.

[8] S. Garg, S. C. Kaul, and R. Wadhwa, "Cucurbitacin B and cancer intervention: Chemistry, biology and mechanisms (review)," *International Journal of Oncology*, vol. 52, no. 1. pp. 19–37, 2018, doi: 10.3892/ijo.2017.4203.

[9] A. P. Saputri, W. A. Wibowo, and B. S. Daryono, "Phenotypical characters and biochemical compound of cucurbitacin melon (Cucumis melo L. 'Gama Melon Parfum') resulted from breeding," in *AIP Conference Proceedings*, Sep. 2020, vol. 2260, pp. 060006(1–7), doi: 10.1063/5.0017615.

[10] U. B. Husnudin, B. S. Daryono, and Purnomo, "Genetic variability of Indonesian eggplant (Solanum melongena) based on ISSR markers," *Biodiversitas J. Biol. Divers.*, vol. 20, no. 10, pp. 3049–3055, 2019, doi: 10.13057/biodiv/d201038.

[11] I. N. Rosyidi and B. S. Daryono, "Phenotypic characters and genetic variations of lurik peanuts (Arachis hypogaea l. var. lurikensis) with inter simple sequence repeat," *Biodiversitas*, vol. 21, no. 2, pp. 629–635, 2020, doi: 10.13057/biodiv/d210227.

[12] V. Portnoy *et al.*, "Next-generation sequencing-based QTL mapping for unravelling causative genes associated with melon fruit quality traits," *Acta Hortic.*, vol. 1151, pp. 9–16, 2017, doi: 10.17660/ActaHortic.2017.1151.3.

[13] M. C. Kyriacou, D. I. Leskovar, G. Colla, and Y. Rouphael, "Watermelon and melon fruit quality: The genotypic and agro-environmental factors implicated," *Sci. Hortic. (Amsterdam).*, vol. 234, pp. 393–408, 2018, doi: 10.1016/j.scienta.2018.01.032.

[14] B. S. Daryono, A. S. Subiastuti, A. Fatmadanni, and D. Sartika, "Phenotypic and genetic stability of new indonesian melon cultivar (Cucumis melo L. 'Melonia') based on ISSR markers," *Biodiversitas*, vol. 20, no. 4, pp. 1069–1075, 2019, doi: 10.13057/biodiv/d200419.

[15] J. Kubola and S. Siriamornpun, "Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (Momordica cochinchinensis Spreng)," *Food Chem.*, vol. 127, pp. 1138–1145, 2011, doi: 10.1016/j.foodchem.2011.01.115.

[16] A. Kumar and R. K. Lal, "The consequence of genotype × environment interaction on high essential oil yield and its composition in clove basil (Ocimum gratissimum L.)," *Acta Ecol. Sin.*, Jun. 2021, doi: 10.1016/j.chnaes.2021.06.002.

[17] H. Fazaeli, H. A. Golmohammadi, S. N. Tabatabayee, and M. Asghari-Tabrizi, "Productivity and nutritive value of barley green fodder yield in hydroponic system," *World Appl. Sci. J.*, vol. 16, no. 4, pp. 531–539, 2012.

[18] Y. L. Chen, V. M. Dunbabin, A. J. Diggle, K. H. M. Siddique, and Z. Rengel, "Development of a novel semi-hydroponic phenotyping system for studying root architecture," *Funct. Plant Biol.*, vol. 38, no. 5, pp. 355–363, 2011, doi: 10.1071/FP10241.

[19] B. Onwuka and B. Mang, "Effects of Soil Temperature on Some Soil Properties and Plant Growth," *Adv. Plants Agric. Res.*, vol. 8, no. 1, pp. 34–37, 2018, doi: 10.15406/apar.2018.08.00288.

[20] C. Zanetti, M. Vennetier, P. Mériaux, and M. Provansal, "Plasticity of tree root system structure in contrasting soil materials and environmental conditions," *Plant Soil*, vol. 387, pp. 21–35, 2015, doi: 10.1007/s11104-014-2253-z.

[21] F. S. Chapin, A. J. Bloom, C. B. Field, and R. H. Waring, "Plant Responses to Multiple Environmental Factors," *Bioscience*, vol. 37, no. 1, pp. 49–57, 1987, doi: 10.2307/1310177.

[22] Z. I. Navia, A. B. Suwardi, T. Harmawan, Syamsuardi, and E. Mukhtar, "The diversity and contribution of indigenous edible fruit plants to the rural community in the Gayo highlands, Indonesia," *J. Agric. Rural Dev. Trop. Subtrop.*, vol. 121, no. 1, pp. 89–98, 2020, doi: 10.17170/kobra-202004061145.

[23] B. S. Daryono and S. D. Maryanto, *Keanekaragaman dan Potensi Sumber Daya Genetik Melon*. Yogyakarta: Gadjah Mada University Press, 2017.

[24] I. N. A. Martiwi, L. H. Nugroho, B. S. Daryono, and R. Susandarini, "Genotypic variability and relationships of sorghum bicolor accessions from Java island, Indonesia based on irap markers," *Biodiversitas*, vol. 21, no. 12, pp. 5637–5643, 2020, doi: 10.13057/biodiv/d211220.

[25] A. F. Irsyad, R. Rindyastuti, T. Yulistyarini, A. S. Darmayanti, and B. S. Daryono, "Genetic variation of agarwood producing tree (Gyrinops versteegii) from Pongkor, Manggarai District, flores Island, Indonesia using ISSR molecular markers," *Biodiversitas*, vol. 21, no. 2, pp. 485–491, 2020, doi: 10.13057/biodiv/d210208.

[26] S. Singh, P. Singh, and J. Singh, "Transposon-based genetic diversity assessment in wild and cultivated barley," *Crop J.*, vol. 5, no. 4, pp. 296–304, 2017, doi: 10.1016/j.cj.2017.01.003.

[27] L. Oktavianingsih, E. Suharyanto, B. S. Daryono, and Purnomo, "Morphological characters variability of taro (Colocasia spp.) in Kalimantan, Indonesia based on phenetic analysis approach," *Sabrao J. Breed. Genet.*, vol. 51, no. 1, pp. 37–56, 2019.

[28] M. Sungkawati, L. Hidayati, B. S. Daryono, and Purnomo, "Phenetic analysis of Curcuma spp. in Yogyakarta, Indonesia based on

morphological and anatomical characters," *Biodiversitas*, vol. 20, no. 8, pp. 2340–2347, 2019, doi: 10.13057/biodiv/d200832.

[29] U. B. Husnudin, Suharyanto, B. S. Daryono, and P. Purnomo, "Variation and non-formal classification of indonesian eggplant (Solanum melongena l.) accessions based on macro and micro-morphological characters," *Agrivita*, vol. 41, no. 3, pp. 544–560, 2019, doi: 10.17503/agrivita.v41i3.2039.

[30] C. Luo, X. H. He, H. Chen, S. J. Ou, and M. P. Gao, "Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers," *Biochem. Syst. Ecol.*, vol. 38, no. 6, pp. 1176–1184, 2010, doi: 10.1016/j.bse.2010.11.004.

[31] E. Zulaika, L. Sembiring, and A. Soegianto, "Characterization and Identification Of Mercury-resistant Bacteria From Kalimas River Surabaya-Indonesia By Numerical Phenetic Taxonomy," *J. Basic. Appl. Sci. Res*, vol. 2, no. 7, pp. 7263–7269, 2012, [Online]. Available: www.textroad.com.

[32] M. K. Fleshman *et al.*, "Carotene and novel apocarotenoid concentrations in orange-fleshed cucumis melo melons: Determinations of β-carotene bioaccessibility and bioavailability," *J. Agric. Food Chem.*, vol. 59, no. 9, pp. 4448–4454, 2011, doi: 10.1021/jf200416a.

[33] B. S. Daryono, S. D. Maryanto, S. Nissa, and G. R. Aristya, "Analisis Kandungan Vitamin Pada Melon (Cucumis melo L.) Kultivar Melodi Gama 1 dan Melon Komersial," *Biog. J. Ilm. Biol.*, vol. 4, no. 1, pp. 1–9, 2016, doi: 10.24252/bio.v4i1.1113.