

Molecular Diversity and Genetic Relationships among Melon (*Cucumis melo* L.) Genotypes Revealed by RAPD Markers

Bambang Supriyanta¹, Endah Budi Irawati¹, Antik Suprihanti², Dessyanto Boedi Prasetyo³,
Fitriani Ramadani¹, Azizah Ridho Ulilalbab¹,

¹ Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Yogyakarta, Indonesia

²Department Agribusiness, Universitas Pembangunan Nasional "Veteran" Yogyakarta, Indonesia,

³Department Informatika, Universitas Pembangunan Nasional "Veteran" Yogyakarta, Indonesia

Received : September 08, 2026

Revised : September 16,
2026

Accepted : September 17,
2026

Online : October 7, 2026

Abstract

Melon (*Cucumis melo* L.) is an economically important horticultural crop in Indonesia, and developing superior cultivars requires sufficient genetic diversity and effective germplasm characterization. This study aimed to assess genetic variation among melon genotypes, identify informative RAPD primers to distinguish melon from timun suri and blewah, and characterize genetic relationships among melon genotypes for breeding purposes. A combined field and laboratory study was conducted at the Plant Breeding Laboratory, UPN "Veteran" Yogyakarta. Eight RAPD primers were evaluated using 10 genetic materials comprising eight melon genotypes, timun suri, and blewah. Amplified DNA fragments were scored as binary data and analyzed using NTSYS-pc 2.1 software. The primers generated 69 loci, with 98.86% polymorphism, indicating substantial molecular variation among the evaluated materials. BC-388 and BC-551 showed the highest discriminatory capacity for separating timun suri and blewah from melon genotypes and were subsequently used to assess genetic relationships among the eight melon genotypes. UPGMA analysis separated the melon genotypes into two major groups at a similarity coefficient of 0.69. Cluster 1 comprised DGMC-2-5-16-1-5, DGMD-4-9-3-3-4, Pearl Lady, Dalmatian, and Golden Apollo, whereas Cluster 2 comprised Orange Melody, Silver Light, and Yuki Lady; both clusters showed a mean similarity coefficient of 0.77. The advanced breeding lines DGMC-2-5-16-1-5 and DGMD-4-9-3-3-4 showed substantial genetic divergence from several genotypes, indicating their potential as parental materials. Overall, RAPD markers revealed substantial genetic variation and relationships, providing preliminary molecular information for strategic parent selection in melon breeding.

Keywords: *Melon, RAPD, Genetic Variation, Dendrogram, PCR*

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the most important horticultural commodities in Indonesia, with substantial economic value. Consumed fresh and utilized as a raw material in the food and beverage industries, melon is valued for its sweet flavor and nutritional content, including vitamin C, provitamin A, and potassium (Hidzroh, 2021). Although originating from Africa, melon has been widely cultivated throughout Indonesia, resulting in diverse varieties that differ in fruit shape, skin netting, flesh color, aroma, and sugar concentration. This broad phenotypic diversity provides an important resource for melon improvement.

Over the past decade, national melon production in Indonesia has shown considerable fluctuation. Data from BPS (2024) indicated that production reached 150,356 tons in 2014 but declined to 117,794 tons in 2023. Such fluctuations highlight the need for improved cultivars with high yield, desirable fruit quality, broad environmental adaptability, and resistance to biotic and abiotic stresses. Plant breeding therefore plays a pivotal role in developing superior cultivars that

Copyright Holder:

© Bambang, Endah, Antik, Dessyanto, Fitriani, & Azizah. (2026)

Corresponding author's email: bambang.supriyanta@upnyk.ac.id

This Article is Licensed Under:



combine agronomic performance with commercially desirable fruit characteristics. For example, 'Golden Apollo' is characterized by non-netted yellow skin, crisp white flesh, and high sugar content (Huda et al., 2017). Systematic breeding programs involve germplasm collection, characterization, parental selection, controlled hybridization, and progeny evaluation (Syukur et al., 2012). The effectiveness of these processes depends strongly on understanding the genetic diversity and relationships among available breeding materials.

Genetic diversity in melon is particularly important because *C. melo* exhibits extensive variation in morphological, agronomic, and fruit-quality characteristics. Molecular studies have demonstrated that this phenotypic diversity is accompanied by substantial genetic variation. Using RAPD and RFLP markers, Garcia-Mas et al. (2000) demonstrated considerable molecular variation among melon varieties and showed that RAPD markers could differentiate genetically divergent melon types. Similarly, RAPD-based analyses of melon breeding lines revealed genetic relationships that were consistent with pedigree and agronomic information, supporting the use of molecular markers for germplasm management and parental selection (Garcia-Mas et al., 2000). Other studies using RAPD, SRAP, and microsatellite markers have also identified substantial genetic diversity and distinct genetic groupings among melon germplasm (Singh & Singh, 2015). More recent microsatellite-based characterization further demonstrated clear genetic differentiation among cultivated and wild melon groups, emphasizing the importance of molecular characterization for effective germplasm utilization (Mallah et al., 2017).

Molecular markers provide an advantage over morphological characterization because DNA-based variation is less directly affected by environmental conditions. Among molecular marker systems, RAPD has been widely applied for preliminary assessment of genetic diversity because it requires relatively simple procedures and can generate polymorphic DNA profiles without prior sequence information. Previous studies have demonstrated its ability to distinguish melon accessions, identify genetically divergent materials, and reveal relationships among breeding lines (Garcia-Mas et al., 2000; Naznin et al., 2024). RAPD analysis has also been successfully used to identify informative markers and genetic groups within diverse melon germplasm collections.

Despite these studies, genetic relationships among locally developed and commercially relevant melon genotypes used in breeding programs in Indonesia remain insufficiently characterized at the molecular level. In particular, information is limited regarding the molecular differentiation of selected melon genotypes from related local materials such as *timun suri* and *blewah*, as well as the genetic relationships among advanced melon breeding lines and commercial cultivars. Such information is valuable for identifying genetically divergent parents and designing more strategic crossing programs.

Therefore, this study aimed to (1) assess the molecular genetic variation among eight melon genotypes, *timun suri*, and *blewah* using RAPD markers; (2) identify informative RAPD primers capable of differentiating melon genotypes from *timun suri* and *blewah*; and (3) characterize the genetic relationships among the eight melon genotypes based on RAPD profiles to support the identification and strategic selection of potential parental materials for future melon breeding programs.

LITERATURE REVIEW

Genetic diversity within plant populations arises from mutation, recombination, and gene migration, forming the bedrock for effective selection and crop improvement (Sembiring et al., 2015). While traditional characterization relies on morphological markers, these phenotypic traits are frequently influenced by environmental conditions and developmental stages. Consequently, molecular DNA markers have become indispensable tools for direct genetic assessment.

Random Amplified Polymorphic DNA (RAPD) is a PCR-based marker technique that uses short arbitrary oligonucleotide primers (typically 10-mers) to amplify random genomic regions without prior DNA sequence knowledge (Gusmiaty et al., 2021). RAPD offers key advantages, including technical simplicity, low cost, small sample requirement, and high efficiency in detecting polymorphism across close accessions (Syukur et al., 2012).

According to population genetics principles, allele and genotype frequencies in random-mating populations remain stable across generations under Hardy-Weinberg equilibrium in the absence of selection, mutation, or migration (Syukur et al., 2012). Assessing changes in heterozygosity and allelic distribution across advanced selection generations enables plant breeders to track genetic fixation, evaluate population uniformity, and select optimal parents for maximum heterosis in crossbreeding programs (Rahayu & Handayani, 2010).

RESEARCH METHOD

Research Location & Materials

This study was conducted at the Plant Breeding Laboratory, Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Yogyakarta, located at Jl. SWK Ring Road Utara No. 104, Condongcatur, Sleman, Special Region of Yogyakarta.

Plant materials consisted of 10 genotypes: melon inbred lines DGM-2-5-16-1-5 (M1) and DGMD-4-9-3-3-4 (M2), commercial melon cultivars Dalmatian (M3), Golden Apollo (M4), Pearl Lady (M5), Orange Melody (M6), Silver Light (M7), Yuki Lady (M8), timun suri cultivar 'Tisuri' (TS), and blewah cultivar 'Blaster F1' (BL). Laboratory reagents included Geneaid Genomic DNA Mini Kit (Plant), 1X TBE buffer, OnePCR™ Plus Master Mix, agarose, DNA ladder marker, GeneDirex Novel Juice staining reagent, loading dye, sterile aquabidest, and 8 RAPD primers (PMAR, BC-388, BC-551, OPA-07, OPAK-16, OPAX-16, OPAJ-18, OPD-13).

Experimental & Laboratory Procedure

A combined field and laboratory experimental design was used. Ten genotypes were grown (1 plant per genotype) for leaf sample collection. Genomic DNA was extracted from young leaves using the Geneaid kit. PCR reaction mixtures were prepared using OnePCR™ Plus Master Mix and amplified in a thermal cycler using RAPD-specific profiling. PCR products were separated by horizontal agarose gel electrophoresis in 1X TBE buffer and visualized under UV illumination using the Uvitec Cambridge gel documentation system.

Data Scoring & Statistical Analysis

Amplified DNA fragments were scored as binary data: present (1) or absent (0). Data matrix processing was conducted using NTSYS-pc version 2.1 software. Genetic similarity coefficients were computed using the DICE/ Jaccard index, and dendrograms were constructed via the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering. Allele frequencies, genotype frequencies, and heterozygosity levels were calculated to evaluate population genetic structure and Hardy-Weinberg equilibrium.

FINDINGS AND DISCUSSION

PCR amplification across the 10 cultivars generated a total of 69 loci, of which 98.86% showed polymorphic DNA bands. Table 1 summarizes the overall DNA fragment profiles generated by the 8 RAPD primers.

Table 1. DNA fragments produced by 8 RAPD primers across 10 cultivars of melon, timun suri, and blewah

No	Primer Code	Fragment Size Range (bp)	Total Bands	Polymorphic Bands	Polymorphism (%)
1	PMAR	550 – 750	3	3	100.0%
2	BC-388	400 – 1400	12	11	91.7%
3	BC-551	400 – 1600	6	6	100.0%
4	OPA-07	280 – 900	7	7	100.0%
5	OPAK-16	550 – 1400	10	10	100.0%
6	OPAX-16	400 – 1300	8	5	62.5%
7	OPAJ-18	250 – 1800	10	10	100.0%
8	OPD-13	300 – 1000	12	12	100.0%
Total / Average			68	64	98.86%

As detailed in Table 2, primers PMAR, OPA-07, OPAK-16, OPAJ-18, and OPD-13 exhibited 100% polymorphism, while OPAX-16 demonstrated the lowest polymorphic rate at 62.5%. Primers with polymorphic rates exceeding 50% are considered effective for resolving genetic variation (Huda et al., 2013).

Table 2. Effectiveness evaluation of RAPD primers for cultivar separation

Primer	Polymorphism (%)	Discrimination Efficacy	Primary Marker Features
BC-388	91.7%	High (Recommended)	Separates melon cultivars from TS & BL at 0.41 similarity
BC-551	100.0%	High (Recommended)	Separates most melons from TS & BL at 0.35 similarity
PMAR	100.0%	Low	Clustered M2 with commercial melons, TS, and BL (1.00 similarity)
OPA-07	100.0%	Low	M6 and M8 closely grouped with TS and BL
OPAK-16	100.0%	Low	Melon genotypes mixed into same cluster as TS and BL
OPAX-16	62.5%	Low	Blewah (BL) clustered with M7 and M8 melon accessions
OPAJ-18	100.0%	Low	M2 grouped with TS and BL
OPD-13	100.0%	Low	M2 and M3 showed high similarity to BL and TS

Although primers such as PMAR, OPA-07, and OPAK-16 displayed 100% polymorphism,

their resulting dendrograms failed to separate melon genotypes from timun suri (TS) and blewah (BL). In contrast, BC-388 and BC-551 proved highly specific and effective in clustering melon genotypes distinctly from TS and BL. Primer BC-388 generated 12 bands (400–1400 bp) and separated the cultivars into two distinct primary clusters at 0.41 similarity: Cluster I (8 melon cultivars) and Cluster II (timun suri and blewah).

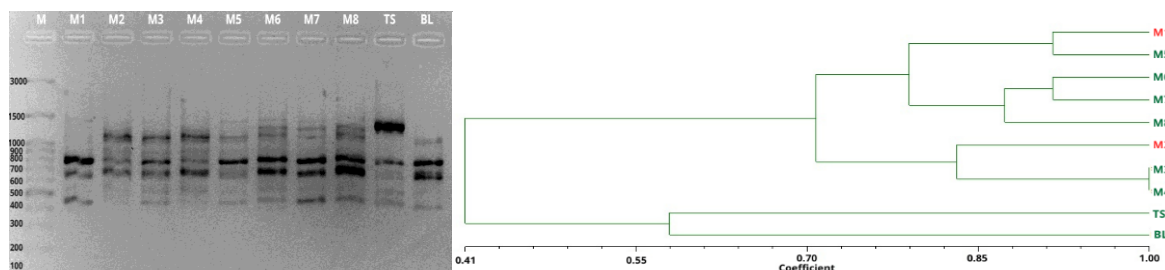


Figure 1. PCR amplification and dendrogram analysis using the BC-388 primer

PCR-RAPD amplification using the BC-388 primer generated 12 DNA fragments ranging from 400 to 1,400 bp, of which 11 fragments were polymorphic. Several specific fragments were observed only in particular samples, including fragments at approximately 400 bp and 1,000 bp in BL (blewah) and a fragment at approximately 420 bp in TS (timun suri). The dendrogram revealed two major groups at a similarity coefficient of 0.41. The first group comprised the eight melon cultivars, whereas the second group consisted of timun suri (TS) and blewah (BL), which were clearly separated from the melon group. These results indicate that the BC-388 primer was effective for assessing genetic variation, as it clearly discriminated the melon cultivars from timun suri and blewah based on their molecular profiles (Figure 1.)

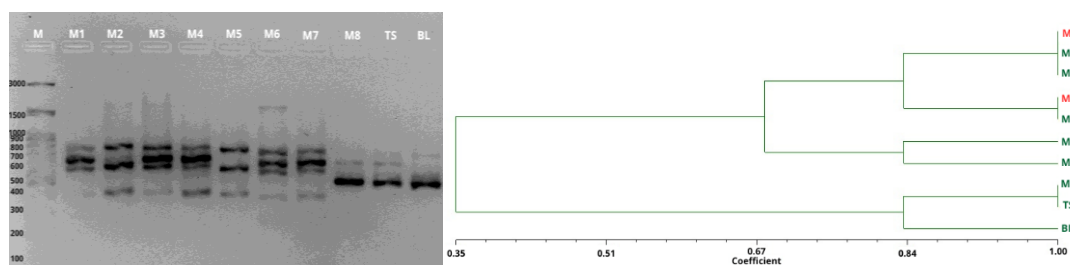


Figure 2. PCR amplification and dendrogram analysis using the BC-551 primer

PCR-RAPD amplification using the BC-551 primer generated six DNA fragments ranging from 400 to 1,600 bp, all of which were polymorphic. The 400-bp fragment was detected in almost all melon genotypes, whereas the 500-bp fragment was detected only in M8, TS, and BL. The 1,600-bp fragment was detected exclusively in M6. The dendrogram revealed two major groups at a similarity coefficient of 0.35. The first group comprised M1–M7, whereas the second group consisted of M8, TS, and BL. These results indicate that the BC-551 primer was sufficiently effective for assessing genetic variation, as it was able to separate most melon genotypes from timun suri and blewah based on their molecular profiles (Figure 2.)

Dendrogram and Cluster Analysis of Melon Cultivars

When analyzing the 8 melon cultivars using the recommended BC-388 and BC-551 primers, similarity indices ranged from 0.69 to 1.00. At a threshold of 0.69, the melon genotypes divided into two main clusters:

- **Group 1 (Mean Similarity = 0.77):** Comprises DGMC-2-5-16-1-5 (M1), DGMD-4-9-3-3-4

(M2), Pearl Lady (M5), Dalmatian (M3), and Golden Apollo (M4). Sub-clustering showed M2 and M5 with 0.88 similarity, while M3 and M4 exhibited identical band profiles (1.00 similarity).

- **Group 2 (Mean Similarity = 0.77):** Comprises Orange Melody (M6), Silver Light (M7), and Yuki Lady (M8), with M6 and M7 sharing 0.88 similarity. (Figure 3.)

Inbred line M1 (DGMC-2-5-16-1-5) derived from M3 × Golden Aurora F1, while line M2 (DGMD-4-9-3-3-4) derived from M3 × M5. The noticeable genetic distance between Group 1 and Group 2 highlights key hybridization targets. In particular, M1 exhibits high genetic distance from M6 and M7, whereas M2 is distant from M6 and M8. Crossing genetically distant lines produces stronger heterosis effects (Rahayu & Handayani, 2010).

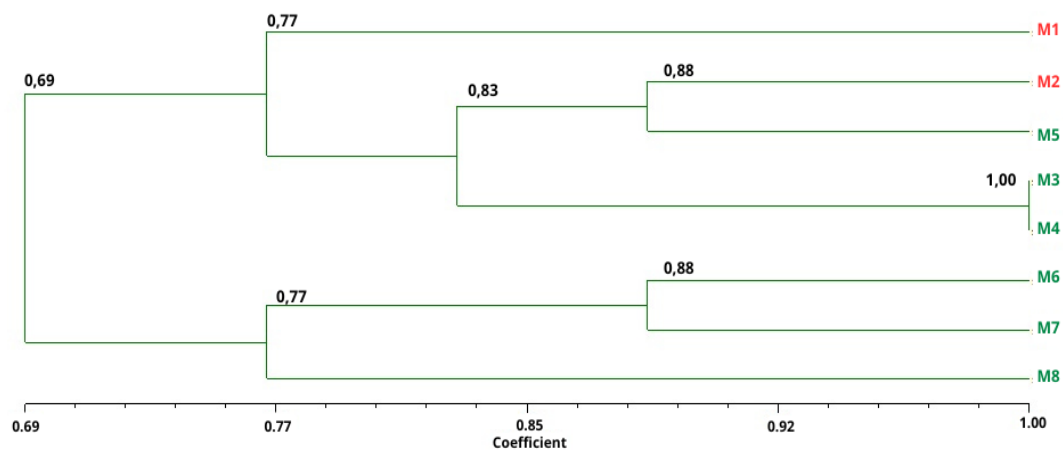


Figure 3. Similarity dendrogram of eight melon cultivars based on molecular characteristics generated using NTSYS-pc 2.1 (Numerical Taxonomy and Multivariate Analysis)

Allele Frequency, Genotype Frequency, and Hardy-Weinberg Equilibrium

Allele Frequency, Genotype Frequency, and Hardy-Weinberg Equilibrium

Table 3. Allele frequency, genotype frequency, and population structure across RAPD primers

Primer	Allele Frequency		Genotype Frequency		
	Dominant (A)	Recessive (a)	AA	Aa	aa
PMAR	0.00	1.00	0.00	1.00	0.00
BC-388	0.61	0.39	0.37	0.48	0.15
BC-551	0.50	0.50	0.25	0.50	0.25
OPA-07	0.00	1.00	0.00	1.00	0.00
OPAK-16	0.00	1.00	0.00	1.00	0.00
OPAX-16	0.60	0.40	0.36	0.48	0.16
OPAJ-18	0.00	1.00	0.00	1.00	0.00
OPD-13	0.00	1.00	0.00	1.00	0.00

Dominant allele frequencies ranged from 0.00 to 0.61, while recessive allele frequencies ranged from 0.39 to 1.00

1.00. Loci tested with primers BC-388, BC-551, and OPAX-16 contained all three genotype classes (AA, Aa, aa) and conformed closely to Hardy-Weinberg equilibrium expectations, demonstrating stability without disturbing selection forces.

Calculations of population heterozygosity revealed a drop from 0.81 in the previous generation to 0.72 in the evaluated generation—a 0.09 (9%) reduction in heterozygosity. This trend confirms that the melon breeding lines are progressing toward genetic fixation and molecular character stability across successive generations.

CONCLUSIONS

This study evaluated the genetic variation of 10 genotypes of melon, timun suri, and blewah using RAPD molecular markers. Primers **BC-388** and **BC-551** were identified as the most effective primers for cultivar identification and grouping. UPGMA dendrogram analysis grouped melon cultivars into two main clusters at 0.69 similarity. Advanced breeding lines **DGMC-2-5-16-1-5** and **DGMD-4-9-3-3-4** demonstrated high genetic stability alongside distinct genetic distances from commercial varieties Orange Melody, Silver Light, and Yuki Lady.

LIMITATIONS & FURTHER RESEARCH

- Limitations: The current investigation evaluated single-plant representatives per genotype using dominant RAPD markers.
- Future Research: Hybridization programs should cross lines DGMC-2-5-16-1-5 and DGMD-4-9-3-3-4 with genetically distant varieties (Orange Melody, Silver Light, Yuki Lady) to maximize heterosis. Further evaluation should incorporate codominant markers (SSR/SNPs) and multi-location field agronomic trials.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Institute for Research and Community Service (LPPM), Universitas Pembangunan Nasional "Veteran" Yogyakarta, for providing financial support for this research project.

REFERENCES

- Badan Pusat Statistik. (2024). *Indonesian fruit production statistics 2014–2023*. <https://www.bps.go.id/>
- Garcia-Mas, J., Oliver, M., Gómez-Paniagua, H., & de Vicente, M. C. (2000). Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. *Theoretical and Applied Genetics*, 101, 860–864. <https://doi.org/10.1007/s001220051553>
- Gusmiaty, H., Restu, M., & Payne, A. (2021). Molecular marker applications in horticultural crop breeding. *IOP Conference Series: Earth and Environmental Science*, 807(1), Article 012015. <https://doi.org/10.1088/1755-1315/807/1/012015>
- Hidzroh, I. (2021). *Melon: Characteristics, nutritional content, and health benefits*. Kanisius.
- Huda, N., Syukur, M., Trikoesoemaningtyas, & Yuniarti, R. (2013). Genetic diversity analysis of melon (*Cucumis melo* L.) using RAPD markers. *Jurnal Agronomi Indonesia*, 41(3), 223–229. <https://doi.org/10.24831/jai.v41i3.8115>
- Huda, N., Syukur, M., Trikoesoemaningtyas, & Yuniarti, R. (2017). Golden Apollo: A new superior melon variety. *Buletin Plasma Nutfah*, 23(1), 1–8. <https://doi.org/10.21082/blpn.v23n1.2017.p1-8>
- Mallah, O. B., Yaish, S., Shtaya, M., Jamous, R. M., Abu Zaitoun, S. Y., & Ali-Shtayeh, M. S. (2017). Exploring genetic variations of Palestinian snake melon (*Cucumis melo* var. *flexuosus*) germplasm using RAPD molecular markers. *Biodiversity & Environmental Sciences Studies Series*, 11(1), 1–10.

- Naznin, P. M., Imoh, O. N., Tanaka, K., Sreynech, O., Shigita, G., Sophea, Y., Sophany, S., Makara, O., Tomooka, N., Monden, Y., Nishida, H., & Kato, K. (2024). Analysis of genetic diversity and population structure in Cambodian melon landraces using molecular markers. *Genetic Resources and Crop Evolution*, 71(3), 1067–1083. <https://doi.org/10.1007/s10722-023-01677-7>
- Rahayu, S. N., & Handayani, T. (2010). Genetic diversity and heterosis in melon (*Cucumis melo* L.). *Jurnal Agronomi Indonesia*, 38(2), 137–142. <https://doi.org/10.24831/jai.v38i2.1684>
- Sembiring, S., Syukur, M., Trikoesoemaningtyas, & Yuniarti, R. (2015). Genetic diversity analysis of melon (*Cucumis melo* L.) using SSR markers. *Jurnal Hortikultura*, 25(4), 315–325. <https://doi.org/10.21082/jhort.v25n4.2015.p315-325>
- Singh, B. D., & Singh, A. K. (2015). *Marker-assisted plant breeding: Principles and practices*. Springer. <https://doi.org/10.1007/978-81-322-2316-0>
- Syukur, M., Trikoesoemaningtyas, & Yuniarti, R. (2012). *Plant breeding*. IPB Press.