Genetic Diversity and Molecular Analysis of Hybrid Melon (*Cucumis melo* L.) genotype Based on Simple Sequence Repeats (SSR) Markers

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ABSTRACT
This study was carried out to identify the potential hybrid melon genotypes with analysis of genetic diversity using the Simple Sequence Repeats(SSR) Marker. Information from the results of genetic diversity analysis can be used to find information on the appearance of several potential hybrid varieties and to support data or confirmation from the results of genetic diversity based on morphological tests. The research was conducted at the Laboratory of Biotechnology, Faculty of Agriculture, University of Brawijaya, from February to May 2023. Research activities included taking leaf samples from 5 melon genotypes, DNA isolation, DNA quality testing, and amplification with SSR markers. Five SSR markers were polymorphic in all genotypes, revealing a total of 29 alleles. The five hybrid genotypes tested produced different DNA band lengths that indicated genetic variation or diversity. Based on the results of principal component analysis with R studio, the distribution of the genotypes of the five melon hybrids spread over four different plots. Genotypes that have a wide distribution or are in different plots indicate a distant kinship relationship and a high level of genetic diversity.The results of principal component analysis also show that there are two main components formed with eigenvalues of 1.88 and 1.01 respectively with a cumulative diversity value of 91.31%.

Keywords: *SSR, DNA, Genetic Diversity*

1. INTRODUCTION

Melon (Cucumis melo L.) is a horticultural fruit with high economic value and is in high demand on the market. This is because the melon fruit tastes sweet and delicious, so the general public likes it. Melon plants have a high potential to export to destinations such as Singapore, Japan, France, and several other countries [4]. Melon in Indonesia is also one of the ten fruit commodities with the highest export volume in 2008–2012 [7]. However, conversely, melon production in Indonesia still tends to fluctuate [22]. Based on BPS data, melon production in Indonesia reached 138,177 tons but decreased in 2021, with total production only reaching 129,147 tons [8]. Meanwhile, in 2022, melon production in Indonesia will also experience a decline, with total production reaching only 118,696 metric tons. Increasing production can be done by assembling hybrid varieties. Hybrid varieties are first-generation (F1) offspring resulting from a cross between two inbred lines with different genotypes. Through this crossing, it is expected to produce varieties with better characters, higher yield potential, resistance to disease, or adaptive to the environment. Before releasing hybrid varieties, it is necessary to identify the appearance of several hybrid melon lines by analyzing genetic diversity. Information from the results of genetic diversity analysis can be used to determine the performance of hybrid melon lines that have the potential to be released as hybrid varieties.

Analysis of genetic diversity can be carried out morphologically but has limitations, namely that it is considered less accurate because it is still affected by environmental factors. Therefore, to support the analysis of genetic diversity data, which is carried out morphologically, and to increase accuracy, the assessment of genetic diversity can be carried out using a molecular approach or by conducting DNA analysis. One of the uses of molecular markers is PCR-based molecular markers, such as simple sequence repeat (SSR) markers. SSR is a microsatellite marker that includes codominant markers and has very informative and valuable properties for population genetic studies and kinship. SSR is a molecular marker in the form of repeated and sequential dinucleotide to tetranucleotide sequences

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[21]. SSR is a short sequence of about 2 to 4 repeated nucleotides that are abundant in the genomes of eukaryotic organisms [30]. SSR markers are considered more effective because they are not affected by environmental factors, are codominant, have a high level of polymorphism, and are very informative, so they can be used for genetic diversity analysis. Genetic diversity analysis has the potential to indicate variations in traits between the several genotypes tested. This high diversity can increase the possibility of the appearance of new and unique traits in genotypes so that they can be used to meet the requirements for the release of new varieties, which must have dictinctiveness. Information on genetic diversity based on these molecular markers can be used to find information on the appearance of several potential hybrid varieties as well as supporting data or a form of confirmation from the results of genetic diversity tests conducted morphologically.

2. MATERIAL AND METHOD

This study was carried out in Laboratory Biotechnology, Faculty of Agriculture, University of Brawijaya

2.1. Plant Material

This study used 5 hybrid melon genotypes which are a selected genotype.

Table 1 Genotype on this study

No	Genotype	Code
1	ACD211303 (A) x ACL221402 (H)	AH
2	ACD211254 (B) x ACL221402 (H)	BH
3	ACD231380 (D) x ACD211254 (B)	DB
4	ACD231380 (D) x ACD211362 (C)	DC
5	ACD231265 (E) x ACD211362 (C)	EC

2.2. DNA Isolation

Melon plants that are 4 WAP can be subjected to a sampling process for DNA isolation. The sampling process was carried out when the melon plants were 4 weeks after planting on the third, fourth, or fifth leaves from the apical. Sampling is carried out with the criteria that the leaves were fresh and not affected from disease. The DNA isolation process was carried out by using the Viogene Plant Mini Kit. Tools used in the process of DNA isolation includes micropipette, spatula, pipette tip, microcentrifuge, 1.5 microcentrifuge tubes, mortars, measuring cups, freezers, vortexes, ovens and analytical balances

2.3. DNA Quality Test

DNA quality testing was carried out by observing isolated DNA bands through electrophoresis and visualization with gel doc. The tools used in the DNA quality testing process include machines electrophoresis, Doc Instrument gel, micropipette, oven, electrophoretic tray and comb. The materials used in the DNA quality test process include distilled water, agarose gel, TAE buffer, loading dye and Gelred.

2.4. PCR Processing using Simple Sequence Repeats Markers

Five primer were used to generate the SSR markers that are CMAG59, CMBR120, CMCT505, CSWCT22A, and CMTA134a. The DNA amplification process was carried out using the Nzytaq II Green mastermix protocol. The amplification of PCR process consists of denaturation, annealing, and extension stages. The denaturation step was carried out for 1 cycle at 95^{o}C for 3 minutes. Then denatured again at 94^{o}C for 30 seconds for 35 cycles. Annealing was carried out at temperatures ranging from 46.3-52^{o}C for 30 seconds and the extension stage at 72^{o}C for 1 minute for 35 cycles. The next stage is the final extension stage at 72^{o}C for 5-10 minutes with 1 cycle. Then the amplification results can be used immediately or can be stored at 20^{o} .

Table 2. Primer SSR that used on this study

No	*Primer*	*Sequence*	PIC	Size of Bands (bp)	Reference
1	CMAG59	F : TTGGGTGGCAATGAGGAA R: ATATGATCTTCCATTTCCA	0,501	131-141	[28]
2	CMBR120	F : CTGGCCCCCTCCTAAACTAA R: CAAAAAGCATCAAAATGGTTG	0,648	167-173	[34]
3	CMCT505	F: GACAGTAATCACCTCATCAAC R: GGGAATGTAAATTGGATATG	0,71	210	[10]
4	CSWCT22A	F: GGGAGTATCGAAACAAAAGC R: TTCTGATCAACGACGAAGTAA	0,654	334	[29]
5	CMTA134a	F : ACGTGCTTCAGTAAACATG R: CCGACATTGAAAACCAACTTC	0,64	150-162	[19]

2.3. Data Analysis

Data analysis was performed by calculating the number of polymorphic alleles and the Polymorphism Information Content (PIC) value. The amplified DNA band length can be observed through the process analysis using gel analyzer software. Next DNA band the scoring process is carried out by changing the DNA bands into binary data with a score (1) for visible bands and (0) for invisible bands. On each sample will also be observed for the proportion of polymorphism and band length DNA. The number of polymorphic alleles was obtained from the visible bands on the geldoc. Polymorphism is a band condition that appears at a certain size and does not appear in other samples [2]. Meanwhile monomorphic is a DNA band that appears at a certain size that is present in all samples so that there are no variations in the size of the DNA bands. The PIC value is intended to determine the strength or informative level of a primer used. PIC value can be known by using the formula :

$$PIC = 1 - \sum_{j=1}^{n} p_{ij}^2$$

with Pij being the j-th allele frequency for the i-marker[5].

PIC values > 0.5 indicate very informative primers, PIC values ranging from 0.25-0.5 are classified as moderate, and PIC values < 0.25 are categorized as low [18]. Data were analyzed using principal component analysis (PCA) to determine the distribution of genotypes and variables that contribute to diversity. The number of main components formed is marked with an eigenvalue > 1 while the variables that contribute to the main component are marked with a factor loading value > 0.5[3] . The main component analysis process is carried out using R studio software. Data from DNA analysis in the form of DNA bands were analyzed using R studio software and would produce a quadrant biplot for principal component analysis [16].

3. RESULT AND DISCUSSION

3.1. DNA Quality Test

Analysis of genetic diversity using SSR markers is strongly influenced by the quality of the DNA from the DNA isolation process. Good DNA quality is indicated by the condition of the DNA band pattern which is not shaded and looks clear or bright. The brighter or clearer the DNA bands that appear indicate the high concentration of pure DNA obtained from the DNA isolation process.

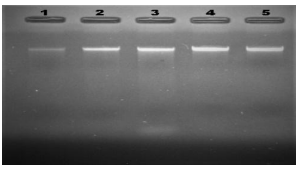

Figure 1 DNA quality test of five hybrid melon genotypes

One of the DNA isolation process's successes was the appearance of clear and unshaded electrophoretic bands. The results of this study indicate that the condition of the DNA bands isolated from the five genotypes shows DNA bands with clear, thick, and unshaded conditions. DNA bands with clear, bright, and non-smear conditions indicate the success of the isolation process in obtaining pure DNA with high concentrations and minimal contaminants from several particles such as RNA, proteins to polysaccharides which can reduce the purity of DNA [1]. One of the successes of the DNA isolation process is influenced by the leaf sampling process. Selecting samples free from fungal infection is intended to avoid contamination from nucleic acids in fungi or viruses which can reduce the purity of DNA [20]. The use of isolation kits also influences success in the DNA isolation process. Isolation kits are considered to minimize contamination from several other particles resulting in high concentrations of DNA[6].

Impure DNA conditions or contamination with other particles can interfere with the primary attachment process at the amplification stage. Other particles, such as polysaccharides, can make DNA solutions thick and sticky and can interfere with the activity of restriction enzymes and Taq Polymerase. The presence of contaminants from other particles can cause the condition of the DNA bands to become shadowed or smeared. Contamination can be caused by cell components that are not lysed, resulting in a protein that is not completely degraded [26]. The quality of DNA isolated from DNA plays an essential role in amplification [25]. DNA that has good quality can be used to utilize molecular markers or sequencing processes. Therefore, the process of DNA isolation is an essential step before carrying out the amplification process.

3.2. Genetic diversity based on Simple Sequence Repeats Marker

The PCR process using the five primers showed polymorphic results in 5 genotypic samples as shown in Tabel 3 and figure 2

Tabel 3. Allele variation and polymorphic information content (PIC) used to assess the genetic diversity on 5 selected melon hybrid genotypes

Primer	Size (bp)	Number of allele	PIC	Polymorphism (%)
CMAG59	68, 82, 87, 107, 117	5	0,8	100%
CMBR120	136, 167, 173, 176, 176	4	0,72	100%
CMCT505	170, 172, 174, 185, 202	5	0,8	100%
, CSWCT22A	211, 225, 239, 242, 255	5	0,8	100%
CMTA134a	151, 163, 168, 173, 174, 308, 328, 334, 338, 341	10	0,6	100%

The difference in the length of the DNA bands indicates the presence of genetic variation and the diversity of the genotypes tested. Genetic variation between genotypes could be known from the differences in pattern, number, and size of DNA bands amplified in each primer used [14]. The results of DNA amplification using the five primers resulted in a polymorphism level of 100%. The resulting polymorphism in the amplification process indicates the existence of allelic diversity between genotypes, which influences the determination of genetic diversity[5]. The appearance of these polymorphic bands indicates differences in each genotype's genetic characters, allowing for differences in phenotypic characters. The emergence of these genetic variations is because the five genotypes come from crosses of different parents so they have the potential to have genetic variations with different traits. Genetic variation or diversity allows various combinations of traits to be obtained. Crosses between two parents who have genotypic differences or large genetic distances have the potential to obtain the desired genetic combination [31].

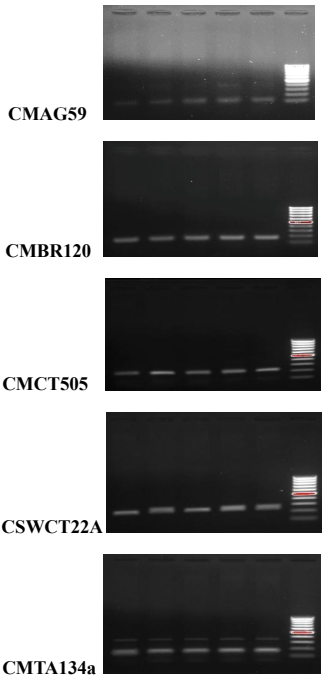

Figure 2 DNA polymorphism in the five hybrid melon genotypes based on SSR markers

The electrophoresis results from the PCR process are influenced by the selection of primers used in the SSR method. The appearance of various alleles in each primer indicates the suitability of the primer with the sample being tested. The appearance of the allele in the primer used indicates a primer that is complementary to the target DNA[2]. The corresponding primers for certain genotypes are also characterized by high PIC values. PIC indicates a primary informativeness level used. PIC values above 0.5 indicate that a primer is very informative [18]. Primers that have very informative PIC values indicate the high ability of these primers to identify a genotype [9]. The PIC values and the appearance of alleles in this study were different from the primary references in previous studies. Differences in DNA band length and PIC values may occur in this study. The diversity of genotypes used in the study, which gave rise to DNA band lengths that were different from references in previous studies [15].

The annealing temperature in the PCR process affects the process of attaching the primer to the DNA template. The optimal annealing temperature can be calculated based on the Tm value or melting temperature and can be used up to 5oC below the melting temperature [13]. One of the other factors that might affect the results of CMAG59 primers which show thin DNA bands is due to the less-than-optimal setting of the annealing temperature. Annealing temperature that is too high can interfere with the primary attachment process [27]. It can cause the formed band to become thin and less clear. Meanwhile, an annealing temperature that is too low can cause the primer to stick to a non-specific attachment site resulting in the amplification of unwanted locus fragments [24]. The success of the PCR process is also influenced by several factors, such as the selection and suitability of the primer, the composition of the buffer solution, the number of reaction cycles, the condition of the dNTP, the DNA template, the enzymes used, and the contamination of the DNA.

3.3. Principal Component Analysis

The results of principal component analysis also show that there are two main components formed with eigenvalues of 1.88 and 1.01 respectively with a cumulative diversity value of 91.31% (Tabel. 4). The number of main components formed is influenced by eigenvalues with more than one eigenvalue forming one main component [23]. PC1 or the first component formed generally has the highest diversity among the other main components, so it is

considered important in genotypic grouping [33]. PC1 and PC 2 have a cumulative variability of 91.31%, which means that the two main components can represent 91.31% of the total variability.Based on Tabel 4. The highest loading factor value on PC1 was obtained on the CMAG59 primer with a value of 0.48. The highest loading factor value on PC2 was obtained on the CMCT505 primer with a value of 0.62. The higher the value of the loading factor indicates the higher the influence of main component formation. Variables that have factor loading values > 0.5 indicates that the variable has the maximum contribution in determining diversity [3]. The variable on PC1 does not have a loading factor value better than 0.5. That matter indicates that no variables contribute significantly or do not contribute maximally in determining diversity. On PC2, some components or variables have factor loading values > 0.5, namely the CMCT505 primer. This indicates that the CMCT505 primer contributes to the maximum on PC2

Tabel 4. Principal Component Analysis Result

No	Primer	PC1	PC2
1	CMAG59	0,48	0,14
2	CMBR120	0,42	-0,56
3	CMCT505	0,40	0,62
4	CSWCT22A	0,47	0,26
5	CMTA134a	0,45	-0,46
	Eigenvalue	1,88	1,01
	Variability (%)	70,93%	20,39%
	Cumulative variablity (%)	70,93%	91,31%

Through the results of principal component analysis using R studio it can be seen about the distribution of the five genotypes based on the five primers used which are visualized in Figure 3. Code 1 indicates the AH genotype, code 2 indicates the BH genotype, code 3 indicates the DB genotype, code 4 indicates the DC genotype, and code 5 denotes EC genotype. Meanwhile P1 denotes CMG59 primer, P2 denotes CMBR120 primer, P3 denotes CMCT505 primer, P4 denotes CSWCT22a primer, and P5 denotes CMTA134a primer.

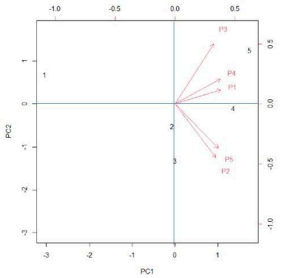

Figure 3 Distribution genotypes based on PCA

In the principal component analysis biplot shown in Figure 3, it can be seen that the AH genotype is located in plot II, the BH genotype is located in plot III, the DC genotype is located in plot IV, and the EC genotype is located in plot I. Meanwhile, the DB genotype is located between plot III and plot IV. Based on the principal component analysis, it can also be seen that the five primers used produce different vector lengths and distributions in different plots. The distribution of genotypes far from the center point indicates that the genotype has a reasonably high diversity value compared to the genotypes close to the center point. Genotypes with a relatively close distribution to one another indicate similarity or close genetic distance between the two genotypes [32]. Genotypes in the same quadrant indicate a close kinship and genotypes in different quadrants at an angle of 90^o indicate distant kinship [12].

An even distribution of genotypes in the four plots indicates genetic diversity in the five genotypes. This genetic diversity has the potential to indicate variations in traits between the several genotypes tested. This high diversity can increase the possibility of the appearance of new and unique traits in genotypes so that they can be used to meet the requirements for the release of new varieties, which must have dictinctiveness. A new variety must have the characteristics of distinctiveness, uniformity, and stability (DUS).The results of molecular-based genetic diversity can be related to the morphological assessment of genetic diversity. Morphological genetic diversity assessment supported by molecular-based genetic diversity analysis can be used to determine genotypes that have the potential to be released as hybrid varieties.

The results of the molecular-based genetic diversity analysis can be used to confirm the genetic diversity tests carried out morphologically. Based on previous research conducted [17], it can be seen that the AH and DC genotypes have differences in all quantitative characters, and the BH and DB lines have the most similarities in quantitative characters. Plants with similar characters indicate a kinship relationship, meaning that the closer the kinship is, the more similarities some characters have. Based on the molecular tests carried out, it can be seen that there is conformity with the results of the morphological tests carried out [20]. The results of DNA analysis showed that the AH and DC strains had a wide distribution and were in different quadrants. Based on DNA analysis, it can be seen that the BH and DB lines have close genotypic distribution points. This indicates that BH and DB have a close kinship relationship, as indicated by the morphological test results between the two genotypes, which show the most similarities in quantitative characters, namely as many as six quantitative characters. In addition, based on the PCR process using the CMBR120 primer, it can be seen that the BH and DB genotypes gave rise to DNA bands with the same length of 176 bp. The appearance of DNA bands of the same size indicates a potential trait similarity and a kinship relationship between the two genotypes tested[11]

4. CONCLUSION

Analysis of genetic diversity based on SSR markers using 5 primers resulted in the appearance of various DNA bands. Five SSR markers were polymorphic in all genotypes, revealing a total of 29 alleles. The five hybrid genotypes tested produced different DNA band lengths that indicated genetic variation or diversity. Principal component analysis showed the formation of 2 main components which played a role in the distribution of the five hybrid melon genotypes. The two main components have eigenvalues of 1.88 and 1.01 with a cumulative diversity of 91.31%. Through principal component analysis it can be seen that the five genotypes have genotype distribution spread over 4 different plots. AH genotype is located in plot II, BH genotype is located in plot III, DB genotype is located between plot III and plot IV, DC genotype is located in plot IV, and EC genotype is located in plot I. Genotypes that have a wide distribution or are in different plots indicate a distant kinship relationship and a high level of genetic diversity

AUTHORS' CONTRIBUTIONS

AFIFUDDIN LATIEF ADIREDJO Supervise the research, writing the manuscript, determine experimental design. RADIN BAYU PUTRA AJIDIN writing the manuscript, analyse data, Laboratory work.

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REFERENCES

[1] Aboul-Maaty, N.A.-F., and H.A.S. Oraby. 2019. Extraction of high-quality genomic DNA from different plant orders applying a modified ctab-based method. Bull. Natl. Res. Cent. 43(1): 1–10. doi: 10.1186/s42269-019-0066-1

[2] Acquaah, G. 2012. Principles of plant genetics and breeding. Wiley-blackwell, Maryland

[3] Agustina, N.I., and B. Waluyo. 2017. Diversity of morpho-agronomic characters and large chili (*Capsicum annuum* L.) lines. J. Agro 4(2): 120–130. doi: 10.15575/1608.

[4] Apriyanti, L., A. Setiadi, and S. Santoso. 2021. Analysis of melon export volume forecasting at PT Bumi Sari Lestari Temanggung, Central Java. J. Ekon. Pertan. and Agribisnis 5(4): 1051–1058. doi: 10.21776/ub.jepa.2021.005.04.8

[5] Ashraf, H., A.M. Husaini, M. Ashraf Bhat, G. Parray, and S. Khan. 2016. SSR based genetic diversity of pigmented and aromatic rice (Oryza sativa L.) genotypes of the western Himalayan region of India. Physiol. Mol. Biol. Plants 22(4): 547–555. doi: 10.1007/s12298-016-0377-8

[6] Azizah, N. N., M. N Mazieda., D. Listyorini., and D. Dahlia. 2014. Optimization of DNA Isolation and Purification of Petunia Hybrida Series Rose Picotewith Geneaid Isolation Kit. In Proceeding Biology Education Conference: Biology, Science, Enviromental, and Learning. Vol. 11(1):273-278

[7] BPS. 2013. Export foreign trade statistics Volume 1. Jakarta

[8] BPS. 2022. Production of fruit crops 2021. Jakarta

[9] Feng, S., R. He., J. Lu., M. Jiang, and X. Shen. 2016. Development of SSR markers and assessment of genetic diversity in medicinal Chrysanthemum morifolium cultivars. Front. Genet. 7(JUN): 1–11. doi: 10.3389/fgene.2016.00113

[10] Hassan, M.Z., A.H.K. Robin, M.A. Rahim, S. Natarajan, and H.T. Kim . 2018b. Screening of melon genotypes identifies gummy stem blight resistance associated with Gsb1 resistant loci. J. Plant Biotechnol. 45(3): 217–227. doi: 10.5010/JPB.2018.45.3.217

[11] Hazmy, Z.D., Ardiarini, N., Respatijarti., Damanhuri., and Adiredjo. 2018. Phenotypic and molekuler marker analysis of F1 population derived from crossing of gogo-dryland x paddy-field rice varieties. Biosience Research. Vol 15(3): 1952-1961

[12] Hetharie, H., S.H.T. Raharjo, and D.E. Jambormias. 2019. Clustering of sweet potato clones based on cluster analysis, principal components and biplots of morphological characters. J. Agron. Indones. (Indonesian J. Agron. 46(3): 276–282. doi: 10.24831/jai.v46i3.18215

[13] Hidayati, H., E. Saleh, and T. Aulawi. 2016. Identification of BMPR-1B (Bone Morphogenetic Protein Receptor IB) gene diversity in Arabic chickens, native chickens and laying hens using PCR-RFLP. J. Peternak. 13(1): 1. doi: 10.24014/jupet.v13i1.2383

[14] Hidzroh, F., and B.S. Daryono. 2021. Character uniformity and stability of melon (*Cucumis melo* L. 'tacapa gold') based on phenotypic characters and inter-simple sequence repeats. Biospecies 14(2): 11–19

[15] Hu, J., L. Gao, Y. Xu, Q. Li, and H. Zhu. 2019. Microsatellite markers reveal genetic diversity and relationships within a melon collection mainly comprising asian cultivated and wild germplasms. Biomed Res. Int. 2019. doi: 10.1155/2019/7495609

[16] Jollife, I.T., and J. Cadima. 2016. Principal component analysis: A review and recent developments. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 374(2065). doi: 10.1098/rsta.2015.0202

[17] Kylyfe, D. 2022. Uniformity test of five strains of F1 melon (*Cucumis melo* L.) Based on Agronomic and Morphological Characteristics. Thesis. Brawijaya University.

[18] Ladjao, H.E., R. Sjahril, and M. Riadi. 2019. Genetic diversity of 22 accessions of local rice toraja Utara based on simple sequence repeats (SSR) markers. J. Bioteknol. Biosains Indones. 5(2): 230. doi: 10.29122/jbbi.v5i2.3031.

[19] Molla, M.R., I. Ahmed, S. Rahman, M.A. Hossain, and M.A. Salam. 2017. Genetic diversity among muskmelon (*Cucumis melo* L.) germplasm in Bangladesh as revealed by microsatellite markers. African J. Agric. Res. 12(44): 3203–3213. doi: 10.5897/ajar2017.12617

[20] Nur Huda, I. 2013. Analysis of Genetic Variation of Melon (*Cucumis melo* L.) Cultivar of gama basket melon using random amplified polymorphic DNA method. Biog. J. Ilm. Biol. 1(1): 41–50. doi: 10.24252/bio.v1i1.446

[21] Pasaribu, A., and S. Agustina Putri. 2017 Preliminary analysis of the molecular diversity of oil palm (*Elaies guineensis* Jacq.) used five SSR primers (Simple Sequences Repeats).. J. Pertan. Trop. 4(1): 47–56

[22] Pradipta, A., and M. Firdaus. 2015 Competitiveness position and factors affecting fruit exports in Indonesia... J. Manaj. Agribisnis 11(2): 129–143

[23] Rif'atunidaudina, R., Sobir, and Awangmaharijaya. 2019. Diversity of potential legume vegetable genetic resources in Indonesia based on ISSR molecular markers. J. Hort. Indones. 10(3): 161–172

[24] Royani, J.I., O.F.B. Sinaga, K.N. Aliyah, D. Hardianto, and T. Agustina. 2022. Screening of simple sequence repeats (SSR) primers from mutated Indigofera zolligeriana Miq plants. IOP Conf. Ser. Earth Environ. Sci. 1114(1). doi: 10.1088/1755-1315/1114/1/012106

[25] Selak, G.V., A.B. Arbeiter, J. Cuevas, S. Perica, and P. Pujic. 2021. Seed paternity analysis using ssr markers to assess successful pollen donors in mixed olive orchards. Plants 10(11): 1–16. doi: 10.3390/plants10112356

[26] Setiaputri, A.A., G.R. Barokah, R.D. Arbajayanti, N. Fabella, and R.M. Pertiwi, et al. 2020. Comparison of DNA isolation methods on fresh and processed fishery products. J. Pengolah. Has. Perikan. Indones. 23(3): 447–458

[27] Setyawati, R., and S. Zubaidah. 2021. Optimization of Primary Concentration and Annealing Temperature in Detecting the Leptin Gene in Ongole (PO) Cattle Using Polymerase Chain Reaction (PCR). .Indones. J. Lab. 4(1): 36. doi: 10.22146/ijl.v4i1.65550

[28] Singh, D., D.I. Leskovar, S.P. Sharma, N.K. Sarao, and V.K. Vashisht. 2020. Genetic diversity and interrelationship among Indian and exotic melons based on fruit morphology, quality components and microsatellite markers. Physiol. Mol. Biol. Plants 26(5): 985–1002. doi: 10.1007/s12298-020-00814-1

[29] Singh, S., S. Pandey, R. Raghuwanshi, V. Pandey, and M. Singh. 2015. SSR analysis for fruit and quality characters in infra-specific mapping population of melon. Indian J. Agric. Sci. 85(1): 32–37

[30] Terryana, R.T., K. Nugroho, H. Rijzaani, and P. Lestari. 2018. Characterization of genetic diversity of 27 chili genotypes based on SSR (Simple Sequence Repeat) markers. J. Life Sciences. 17(2): 183–194

[31] Utomo, S. D., K. F. Hidayat., A. Edy., N. Sa'diyah., R. Indriyani., E. Halimaturosidah., and H. Yustina. 2021. Artificial Hybridization of Peanut and Phenotype Character Growth Type, Pod Size, and Number of Seeds per Pod of Hybridized F1 Plants. Journal of Agrotropika. Vol 20(1), 49

[32] Worede, F., T. Sreewongchai, C. Phumichai, and P. Sripichitt. 2013. Genetic diversity analysis of rice cultivars from various origins using simple sequence repeat (SSR) markers. African Journal of Biotechnology 12(26): 4074–4081. doi: 10.5897/AJB2013.12382

[33] Worede, F., T. Sreewongchai, C. Pumichai, and P. Sripichitt. 2014. Multivariate analysis of genetic diversity among some rice genotypes using morpho-agronomic Traits. J. Plant Sci. 9(1): 14–24

[34] Zhang, C., Y. Ren, S. Guo, H. Zhang, and G. Gong. 2013. Application of comparative genomics in developing markers tightly linked to the Pm-2F gene for powdery mildew resistance in melon (*Cucumis melo* L.). Euphytica 190(2): 157–168. doi: 10.1007/s10681-012-0828-4