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Performance of tomato M7 mutant lines and their similarities to the parents based on SSR markers

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Abstract. *Arrufitasari PN, Sutjahjo SH, Wirnas D. 2022. Performance of tomato M7 mutant lines and their similarities to the parents based on SSR markers. Biodiversitas 23: 1239-1245.* Tomato cultivation in the lowlands can be an alternative to increase tomato production so that high-yielding and lowland adaptive varieties are required. This study was aiming at determining the performance of M7 mutant lines and their similarities to the parents based on SSR markers. The experiments were conducted at KP Leuwikopo (250 m asl) and Plant Molecular Biology Laboratory, IPB University, Indonesia. The results showed that the G4 line had the highest productivity among the tested genotypes (36.72 ton ha^{-1}) and significantly differed from all check varieties. The highest number of fruit per plant was 51.67 in the G7 line. On the other hand, G2, G3, G7, G8, and G10 lines had the lowest percentage weight and the number of fruits cracking and were not significantly different from the check varieties (Tora and Mawar). A total of 7 SSR primers detected 87 alleles with a range of 7-15 alleles per locus. The similarity between mutant lines and their parents ranged from 44-69%. The mutant most closely resembled the parent was the G3 line, while the most different based on molecular markers was the G9 line.

Keywords: Dissimilarity, fruit cracking, marker, mutation

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the essential vegetables in fulfilling nutrition and industrial raw materials (Sikder et al. 2013; Aralikatti et al. 2018). Tomato productivity and production in Indonesia fluctuated between seasons and locations (Statistic of Indonesia and Directorate General of Horticulture 2021). Currently, tomato cultivation is generally carried out in the highlands, but the lowlands can be an alternative planting area to increase tomato production. The obstacle to tomato cultivation in the lowlands is that tomato is easy to crack; this phenomenon can reduce productivity and quality. Tomato cultivation in the lowlands (0-250 m asl) needs to be supported by the availability of varieties that are high-yielding and also resistant to fruit cracking.

High-yielding and adaptive varieties in the lowlands can be developed through physical mutations using gamma-ray irradiation followed by the selection of the M2 or M3 generations. Gamma-ray irradiation is the most common physical mutagenic source to produce plant breeding material with high genetic variation. For example, Toni et al. (2013) found that low doses of gamma-ray radiation (2.5-20 Gy) produced varieties with higher average fruit weight, fruit diameter, number of fruits, and productivity.

Based on Law No.13 of 2004 (Ministry of Agriculture 2021a), essentially-derived varieties are varieties resulting from the local variety using the mutation technique so that the variety retains the expression of the essential characteristics of the local variety. However, it can be clearly distinguished from its local variety from the characteristics of the act of descent. A variety is called an essential variety if the characteristics of the local variety can be maintained at least 70%, which means that the resulting variety and its parents have at least 70% similarity.

The experiment of mutant line similarity with the local variety as the parents is necessary to ensure that induction mutation has changed the gene expression and phenotype. The similarity of mutant lines with the parents can be observed based on morphological and agronomical characters and genetically using molecular markers. Similarity tests based on morphological and agronomical characters need to be equipped with a test based on genetic markers because according to Mondini (2009) genetic markers are more accurate and independent of environmental factors.

One of the molecular markers used is SSR (simple sequence repeat). The advantages of SSR markers are high reproducibility, codominant markers, and can detect specific loci. Also, its high polymorphism level and vast spread across all genomes make it the most commonly used marker for the plant breeding program (Aiello et al. 2020). In previous studies, ten of the best M7 mutant lines were resulted from induced mutations and selected for yield improvement and adaptation to the lowlands. Romadhon et al. (2018) reported that these mutant lines have better yield potential than the parents.

Descriptors of quantitative characters, both morphological and agronomic, do not always provide

genetic differences or similarities between genotypes because quantitative characters are influenced by environmental factors (Kwon 2009). Therefore, it is still necessary to carry out a genetic similarity analysis based on molecular markers to support morphological and agronomic character data. Therefore, the research objectives were to obtain information on the yield performance of tomato M7 mutant lines and their similarity with the parents based on SSR molecular markers.

MATERIALS AND METHODS

Genetic materials and study area

The study consisted of two experiments, i.e., a study of the diversity of M7 mutant lines and analysis of similarities between M7 mutant lines and the parents. The genetic materials used for evaluation of the performance of the mutant line consisted of ten M7 mutant lines (M6/Lombok 4/1-3-6, M6/Lombok 1/9-2-8, M6/Lombok 2/2-2- 6, M5/GL2 8-10, M5/Kemir 4-74, M6/Kefamino 6/1-3-7, M6/STBGL 2-3-7, M6/Kudamati 1/1-9-4, M6/STBGL 1-9-4, and M6/Lombok 1/9-5-8) with four check varieties (Tora, Permata, Mawar, and Warda). The second experiment used the same mutant lines and the parents as checks (Lombok 1, Lombok 2, Lombok 4, Kudamati, Wave 2, and STB GL). The first experiment was conducted from September 2020 to March 2021 at the Leuwikopo experiment farm. The second experiment was conducted from August 2021 to October 2021 at the Plant Molecular Biology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB, Bogor, West Java, Indonesia.

Working procedures

The experimental design employed a Randomized Complete Block Design (RCBD) with three replications, and ten plants were observed for each experimental unit. The experimental unit was a plot measuring 5 m x 1 m with a spacing of 50 cm x 50 cm. Observations were made on plant height, fruit weight per plant, number of fruits per plant, percentage weight of fruit cracking, percentage number of fruit cracking, fruit hardness, and productivity.

The experiment was started by sowing the M7 tomato seeds in a tray containing a mixture of soil and manure (2:1). Routine maintenance included foliar fertilization and watering. The seeding was carried out for four weeks in a plastic house. Seedlings were transplanted in the afternoon when the tomato plants had four true leaves. The manure application of 1500 kg ha^{-1} and Urea fertilizer 300 kg ha^{-1}, SP-36 500 kg ha^{-1}, KCl 300 kg ha^{-1} was carried out one week before planting. After that, regular fertilization was carried out every week with 250 mL of NPK fertilizer solution. All the recommended agronomic practices were carried out. Harvesting was carried out when the tomato started to turn reddish.

DNA isolation

Total genomic DNA was isolated from four-week-old fresh leaves by DNA extraction procedure using CTAB (Cetyl Trimethyl Ammonium Bromide) method described by Doyle and Doyle (1990). Next, the mixture in the tube was incubated at 65°C for 30 minutes and homogenized by turning every 5 minutes. Then, 750 µL of chloroform: isoamyl alcohol (24:1) was added into a micro-tube, followed by centrifugation at 12,000 rpm for 15 minutes. A total of 600 µL of the supernatant formed was transferred into a 1.5 mL microtube, then added with 60 µL 3M sodium acetate (pH 5.2) and 600 µL cold isopropanol. The mixture was then left in the refrigerator at -20°C for 1 hour. After that, the mixture was centrifuged at 12,000 rpm for 20 minutes. The supernatant was discarded and the pellet was rinsed with 200 µL of 70% ethanol. Subsequently, centrifugation was carried out for 5 minutes at a speed of 12,000 rpm. The supernatant was discarded, and the cleaned pellets were dried overnight.

DNA amplification

DNA amplification was performed using a PCR machine (Bio-Rad, USA) with the primer sequence information is presented in Table 1. Each sample was amplified in a total reaction of 20 µL containing 20 ng DNA template, 10× buffer (Kapa Biosystems, USA), dNTP mix10 mM (Kapa Biosystems, USA), forward and reverse primers 0.5 µM, and DNA polymerase Taq enzyme (Kapa Biosystems, USA) 5U μL^{-1}. The DNA amplification process began with initial denaturation carried out at 94°C for 4 minutes, followed by 35 cycles of DNA denaturation (94°C, 30 seconds), the primer annealing (55°C, 30 seconds), and the extension (72°C, for 45 seconds). The PCR reaction ended with the final step of base extension at 72°C for 7 minutes.

Table 1. Molecular markers used in this study (Herison et al. 2020)

SSR marker	Forward	Reverse	Ta (ºC)
Sola Pair 1	ATG CAA TAC ACC CTG CGG AA	TGC TAG TGG CCT GTT GAT GG	51
Sola Pair 2	CCA TCA ACA GGC CAC TAG CA	ACG GCT GGA TTG AAG GAA CA	53.1
Solanum	TGC GGA AAT TTT TAT CCA	AAT CAA AGT ACG GAA TAC AAA AA	43.8
Sola 1	CCT GCG GAA ATT TTT ATC CA	AGT GGC CTG TTG ATG GAG TC	50.05
Lyco 4	CCC TTT CAA CGT GAA CGA CT	TCA GCA CCC AAA TCT TCA AA	49.85
Lyco 1	CGG AGT TCT TAA CGC TGC TC	TCA GCA CCC AAA TCT TCA AA	50.15
CACCEL 1i	CTCTAATAGGCAATAGCTCACATGC	GCAGTCTCCCAGAACGTTGTCC	50

DNA visualization/electrophoresis

The gel plates, spacers, and combs were pre-washed with water and ethanol. The electrophoresis technique was carried out on 1% agarose gel in a tank containing 1x TAE (Tris-Acetate-EDTA) buffer. The mixture was poured into the prepared sandwich plate, and the comb was inserted. The results of the PCR-SSR were added with a loading buffer of 2.5 µL for 10 µL of the PCR results. A total of 5 µL of the sample was pipetted into the well. Then, electrophoresis was carried out with a voltage of 90 Volts for 30 minutes. Next, the gel was first washed with ddH_2O then placed and shaken in a tray for 7 minutes. After that, the gel was washed with ddH_2O 2 times. The electrophoretic gel was then observed under UV light in a UV Transilluminator.

Data analysis

Analysis of variance (ANOVA) was performed using the SAS 9.4 program and continued with a post hoc test using the DMRT 5% in the first experiment. Furthermore, the value of the variance components and heritability was calculated based on the separation of the expected mean squares from each source of variance.

Molecular data from the second experiment were analyzed based on the scoring method using DNA bands that appeared on the electrophoresis result on 1% agarose gel. The DNA bands shown on the gel were considered an allele. DNA bands with the same migration rate were assumed as a homolog locus. At the same migration rate, each DNA band was scored 1, while the absence of a DNA band was scored 0. Therefore, the band scoring result is binary data. The DNA band position scoring was performed using the Uvitech software. The scoring data were analyzed using the *Sequential Agglomerative Hierarchical and Nested-Unweighted Pair-Group Method with Arithmetic* (SAHN-UPGMA) on NTSYS version 2.1 (Rohlf 2000). The analysis results were presented in a dendrogram. Subsequently, scoring data, we also analyzed using the *PowerMarker 3.25* software to determine the value of the major allele frequency, genetic diversity, and *polymorphic information content* (PIC) produced by the markers used in this study (Liu and Muse 2005).

RESULTS AND DISCUSSION

Yield components of tomato M7 mutant lines and the check varieties

The analysis of variance showed that the genotype had a significant effect on all the observed characters, indicating differences in the mean values between the genotypes tested. The results showed that the plant height of the M7 mutant line ranged from 63.7 cm (G8) to 127.6 cm (G9), while that of the check varieties ranged from 60.0-121.2 cm. Five M7 mutant lines, i.e., G1, G2, G3, G8, and G10, had plant heights that were not significantly different from the Tora, the shortest check variety. The G4 and G9 lines' plant height was not significantly different from Mawar (Table 2).

Based on plant height, tomato plants are grouped into determinate and indeterminate growth types (Heuvelink 2005). The G3, G4, G5, G6, G7, G8, and G9 lines had an indeterminate growth type, while the G1, G2, and G10 lines had a determinate growth type. The Decree of the Minister of Agriculture No. 084 on the Description of Tomato Varieties (Ministry of Agriculture 2021b), declares that the ideal height for tomato varieties with indeterminate growth type ranged from 100-230 cm, while for determinate growth types, it ranged from 60.90-150 cm. The G1, G2, G4, G6, and G10 lines were candidate varieties with ideal plant height among tested genotypes.

G4 line had the highest mean productivity among the tested genotypes (36.72 tons ha^{-1}) and was significantly different from all check varieties. The other lines had productivity equivalent to the check varieties (Table 2). The character of yield per plant highly affects the productivity of a line (Avdikos et al. 2021). The highest number of fruits per plant was 51.67 in the G7 line. The high yield can be obtained by using superior tomato genotypes adapted to the agro-climate (Sulistyowati et al. 2016).

Table 2. Yield and yield components of tomato M7 mutant lines

Genotype code	Genotype	Plant height (cm)	Yield per plant (g)	Number fruit per plant	Productivity (ton ha^{-1})
G1	M6/Lombok 4/1-3-6)	67.5d	666.6bc	35.69b	21.33bc
G2	M6/Lombok 1/9-2-8)	67.2d	685.9bc	34.1ab	21.95bc
G3	M6/Lombok 2/2-2-6	69.5d	735.2bc	33.9ab	23.53bc
G4	M5/GL 2/8-10	122.5ab	1147.4a	41.8ab	36.72a
G5	M6/Kemir/4-7-4	96.5c	883.9ab	29.9b	28.28ab
G6	M6/Kefamino 6/1-3-7	109.6bc	801.8b	37.9ab	25.66b
G7	M6/STBGL/2-3-7	98.8c	696.0bc	51.67a	22.27bc
G8	M6/Kudamati 1/1-9-4	63.7d	424.7c	22.85b	13.59c
G9	M6/STBGL/1-9-4	127.6a	892.2ab	32.80ab	28.55ab
G10	M6/Lombok 1/9-5-8	65.7d	625.8bc	28.26b	20.03bc
C1	Tora	65.0d	678.4bc	26.40b	21.71bc
C2	Mawar	121.1ab	764.3bc	27.67b	24.46bc
C3	Permata	96.9c	673.7bc	28.56b	21.55 bc
C4	Warda	104.2c	818.3b	24.26b	2618b

Note: Number followed by the same letter in the same column were not significantly different to DMRT 5% level

Table 3. Percentage weight of cracking fruit, percentage number of cracking fruit, and fruit hardness of tomato M7 mutant lines

Genotype code	Genotype	Percentage weight of fruit cracking (%)	Percentage number of fruit cracking (%)	Fruit hardness (kg cm^{-1})
G1	M6/Lombok 4/1-3-6)	34.7ab	10.8c	59.2cd
G2	M6/Lombok 1/9-2-8)	2.3c	4.1c	57.7cd
G3	M6/Lombok 2/2-2-6	12.3bc	6.7c	47.3d
G4	M5/GL 2/8-10	42.3a	37.5a	85.8a
G5	M6/Kemir/4-7-4	35.1ab	50.2a	81.4a
G6	M6/Kefamino 6/1-3-7	39.7a	36.7a	89.6a
G7	M6/STBGL/2-3-7	14.3bc	13.6bc	82.8a
G8	M6/Kudamati 1/1-9-4	6.6c	9.2c	67.8bc
G9	M6/STBGL/1-9-4	48.3a	37.4a	78.1ab
G10	M6/Lombok 1/9-5-8	4.4c	4.1c	57.9cd
C1	Tora	2.7c	3.3c	47.6d
C2	Mawar	52.4a	39.6a	89.3a
C3	Permata	4.1c	5.8c	62.1cd
C4	Warda	45.5a	30.7ab	60.7cd

Note: Number followed by the same letter in the same column were not significantly different to DMRT 5% level

Percentage weight and the number of fruit cracking and fruit hardness are related to fruit cracking resistance. Tora and Permata had a lower percentage weight and the number of fruit cracking and fruit hardness among the four check varieties used, compared to Mawar and Warda (Table 3). The G2, G3, G7, G8, and G10 lines had the lowest percentage of weight and number of fruits cracking among the tested M7 mutant lines and were not significantly different from the check varieties (Tora and Mawar). Tora, Permata, and Warda had the highest fruit hardness and were substantially different from Mawar. Only the G1, G2, G3, and G10 lines had a hardness level equivalent to the Tora, Permata, and Warda. According to Nabil et al. (2012), tomato fruit hardness is influenced by cell wall strength. The weaker the cell wall, the lower the fruit hardness. Several factors affecting tomato fruit hardness are the age of the fruit and environmental factors (Gebregziabher et al. 2021). A high level of fruit hardness can reduce the ability of the fruit skin to stretch, leading the fruit to crack easily (Wang 2021). Xue et al. (2020) reported that the expression of *Solyc02g080530.3 gene* in tomato varieties susceptible to fruit cracking causes an increase in cell wall hardness and inhibits cell wall elongation, which causes the fruit to crack when there is swelling of water absorption.

It is expected that the lower the percentage weight and the number of fruit cracking and fruit hardness level, the more resistant the variety to fruit cracking. Cracked fruit in tomatoes occurs due to changes in the rapid growth rate caused by genetic and environmental factors (Iqbal et al. 2013). According to Ulinnuha et al. (2021), environmental factors influencing fruit cracking are rainfall and high light intensity. Genetic factors include genetic control on aspects of fruit anatomy, fruit growth speed, calcium and boron content, and fruit cell wall strength (Jiang 2019). According to Mustafa et al. (2017), the cracking resistance measured by the fruit cracking index in tomatoes is controlled by two double recessive epistatic genes. Romadhon et al. (2018) reported that the percentage weight of cracking fruit and fruit cracking index was inherited with a high heritability value.

Fruit cracking in tomatoes is grouped into concentric and radial cracking types (UPOV 2011). Romadhon et al. (2017) reported that flat-shaped fruits had a greater incidence of fruit cracking than round ones since flat-shaped fruits have more fruit cavities and less fruit flesh. Based on the research results, the G1, G2, G3, G7, and G10 lines had round fruit shapes with concentric cracking types, while the G4, G5, G6, G8, and G9 lines had flat fruit shape with radial cracking type.

Estimation of variance components and heritability

The difference between the tested genotype's means can be caused by genetic and environmental influences and interactions between genetic and environmental factors. The magnitude of the genetic effect on population diversity is determined by the heritability value. The heritability value needs to be calculated to determine the magnitude of the influence of genetic factors in the inheritance of a trait (Medico 2019).

The values of the variance components, broad-sense heritability, and genetic diversity coefficients are shown in Table 4. The observed character heritability values ranged from 44.0% to 95.3%. All observed characters had high heritability, except for fruit weight per plant. The coefficient of genetic diversity observed in this study ranged from 9.89-26.08% and was classified as high. Mawasid et al. (2019) reported that a high heritability value indicated that this character had the potential to advance selection. The high heritability value and the high coefficient of genetic diversity suggest that genetic factors cause differences in performance between genotypes. It showed that the irradiation mutation used has succeeded in forming diversity. The selected mutant lines have different homozygous alleles so that in the next generation, there is still diversity between lines.

Table 4. Estimates of genetic parameters of tomato M7 mutant lines

Characters	Genetic variance (σ^2g)	Phenotypic variance (σ^2p)	Board-sense heritability (h^2bs) (%)	Criteria	Coefficient of genetic variance (%)
Plant height (cm)	551.83	578.94	95.32	High	9.89
Yield per plant (g)	16541.01	26922.88	61.44	High	23.54
Number of fruit per plant	25.68	58.46	43.93	Medium	24.43
Percentage weight of fruit cracking (%)	322.26	377.41	85.39	High	26.08
Percentage number of fruit cracking (%)	249.66	284.61	87.72	High	19.77
Fruit hardness	206.26	225.92	91.30	High	11.10

Table 5. Allele number, major allele frequency, gene diversity, dan polymorphic information content (PIC) from 21 tomato genotypes

Marker	Allele number	Major allele frequency	Gene diversity	Heterozygosity	PIC
Sola Pair1	11	0.40	0.80	0.90	0.78
Sola Pair2	14	0.43	0.79	0.86	0.78
Solanum	15	0.50	0.73	1.00	0.72
Lyco 1	15	0.44	0.78	0.94	0.77
Lyco 4	14	0.41	0.79	1.00	0.78
Caccel 1i	7	0.33	0.80	0.56	0.77
Sola1	11	0.46	0.75	0.93	0.74
Total	87				
Mean	12.43	0.43	0.78	0.88	0.76

The similarities between the M7 mutant lines and their parents

Gamma-ray irradiation is an induced mutation that can produce essential derivative varieties if the similarity of the resulting variety to the original parent is equal to or more than 70%. The similarity between genotypes can optimally be based on genotype data using molecular data to provide information on the size of the genetic similarity of a genotype with other genotypes (Rouselle et al. 2014). Similarity analysis in this study was carried out based on SSR markers using the primers listed in Table 1; the analysis aimed to determine the level of similarity between the M7 mutant genotype and its parents.

The results of visualization of DNA amplification on gel electrophoresis revealed that all SSR primers produced polymorphisms in the tomato genotypes tested in the study (Figure 1). Seven SSR primers detected 87 alleles, an average of 12 alleles per primer with a range of 7-15 alleles per locus (Table 5). Solanum and Lyco 1 primers produced the highest number of alleles, each capable of detecting 15 alleles, while the lowest was CACCEL1i primer which had the lowest number of alleles, seven alleles. Major allele frequency describes the number of alleles in a population or the total alleles (Gongolee et al. 2016). This study's average major allele frequency was 0.43, with the lowest major allele frequency found in the CACCEL1i primer and the highest allele frequency was in the Solanum primer. The genetic diversity value obtained in this study ranged from 0.73 (Solanum) to 0.80 (Sola Pair 1 and CACCEL1i) with an average of 0.78. The high genetic diversity index illustrates the ability of these SSR markers to differentiate between tested genotypes (Lestari et al. 2021).

The average heterozygosity value in this study ranged between 0.56 (CACCEL1i) and 1.00 (Lyco 4 and Solanum). The heterozygosity value describes the probability that two alleles randomly selected from a population show diversity (Chaerani et al. 2011). The PIC value was used to determine the primer to differentiate between the tested tomato genotypes. The PIC value of each primer in this study showed a relatively high informative level with an average PIC value of 0.76. According to Aguirre et al. (2017), the high mean value of PIC indicates the high genetic diversity of the germplasm tested. The PIC calculation is the number of alleles produced by a primer and the frequency of each allele in the accession set being tested.

Cluster analysis demonstrated that the tested genotypes separated into two main groups at a similarity level of 51%. The first group consisted of 8 genotypes (G1, G3, Lombok 1, Lombok 2, Lombok 4, Kudamati, GL2, STBGL), and the second group consisted of 8 genotypes (G2, G5, G6, G6, G8, G9, G10, G4) (Figure 2). According to Nugroho et al. (2017), genotypes grouped at a similarity level of 51% are considered to have a distant kinship. Therefore, it is suspected that the lines tested in this study using gamma-ray irradiation have undergone genetic changes. Radiation can induce mutations because the irradiated cell will be burdened by high kinetic energy; hence, affecting or changing the chemical reactions of plant cells, which can cause changes in genetic material (Belli et al. 2020). A study by Kim et al. (2021) in M2 plants (2nd generation mutant line) showed that gamma irradiation caused mutations transmitted to the next generation. Gamma irradiation could be considered for the formation of mutant populations in tomatoes.

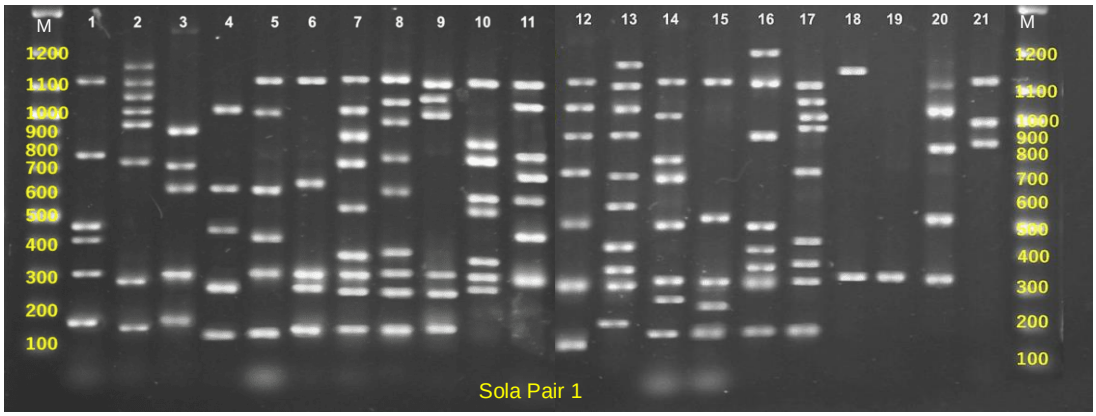

Figure 1. Band pattern of 21 tomato genotypes using Sola Pair 1 primer

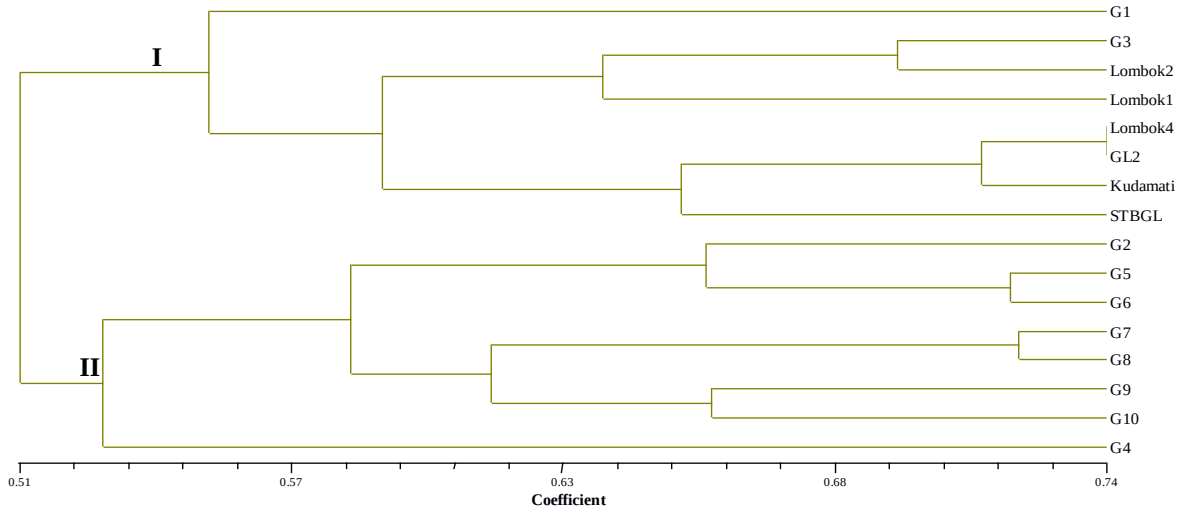

Figure 2. Dendrogram of 10 mutant lines and six parents based on SSR markers analyzed using NTSYS software

Tabel 6. Summary of similarities analysis of M7 tomato mutant lines to the parents

M7 mutant line	Code	Parent	Similarity (%)
G1	M6/Lombok 4/1-3-6)	Lombok 4	55
G2	M6/Lombok 1/9-2-8)	Lombok 1	57
G3	M6/Lombok 2/2-2-6	Lombok 2	69
G4	M5/GL 2/8-10	GL	51
G5	M6/Kemir/4-7-4	Kemir	-
G6	M6/Kefamino 6/1-3-7	Kefamino	-
G7	M6/STBGL/ 2-3-7	STBGL	51
G8	M6/Kudamati 1/1-9-4	Kudamati	47
G9	M6/STBGL/1-9-4	STBGL	44
G10	M6/Lombok 1/9-5-8	Lombok 1	50

The study results indicated that the percentage of similarity between the tested mutants and their parents ranged from 44-69%. The mutant that most closely resembled its parents was the G3 line, while the most distant based on SSR markers was the G9 line (Table 6). The similarity value showed the distance or genetic similarity between the analyzed genotypes. A greater similarity value indicates a closer genetic similarity. Thus, the greater the similarity value, the higher the level of genetic similarity between the tested genotypes. According to Dewi et al. (2016), a genetic distance value of 0 or a genetic similarity value of 1 indicates an absolute genetic similarity between these genotypes.

To conclude, mutant lines tested were not included in the essential derivative group. Hence, further in the registration of the release of varieties, the superiority of these mutant lines was compared to the check varieties determined by the Variety Release Assessment Team referring to the Regulation of the Ministry of Agriculture. No. 38 concerning the Registration of Horticultural Crop Varieties (Ministry of Agriculture 2021c). It also applies to breeding varieties through crosses. Therefore, the G4, G5, and G9 lines were mutant lines with the potential to be released as a high-yielding variety.

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