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Qualitative Characters Diversity of Prentul Kediri Cayenne Pepper Mutant (M1) Resulted from Gamma Ray Mutation Induction

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ABSTRACT

Prentul Kediri cayenne pepper is a local variety that has the potential to be developed into a new superior variety through the breeding of gamma-ray mutations. The purpose of the study is to find out the diversity and degree of similarity in plants of the M1 generation based on qualitative character. The research was conducted in August 2021-February 2022 at the research field, Ketindan Village, Malang City. This study was conducted in a completely randomized block design with three replications. Data was subjected to cluster analysis using the NTSYSpc.2.02i program. The results showed that induction of gamma-ray mutations led to phenotype diversity in several qualitative characters, namely plant habitus, leaf shapes, and fruit shapes, while two other characters, i.e., fruit calyx and the position of flowers remained uniform. The degree of intermutants at each dose of gamma ray mutation is varied. Qualitative characters that remained uniform in the mutant population (M1) were the calyx character of the fruit and the position. In the mutant population (M1), the results of mutations in gamma rays at doses of 100 Gy and 200 Gy were still found in individuals with characters 100% similar to control plants. Meanwhile, in the mutant population (M1), the results of mutations in gamma rays at a dose of 300 Gy were not found to have individuals with characters 100% similar to control plants.

1. INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.) Prentul Kediri variety is one of the popular local commodities for the local community (Rivera *et al.*, 2019). This local variety is characterized by a larger fruit size than ordinary cayenne pepper and a convoluted fruit shape. The advantage of this local cayenne pepper lies in its spicier taste compared to cayenne pepper on the market. Prentul Kediri cayenne pepper is considered to have the potential to be developed as a new superior variety through genetic improvement. The development of new superior varieties can be pursued through plant breeding using mutation induction methods (artificial mutations) using gamma rays.

Plant breeding through mutation induction or artificial mutation is an effort to improve the character or nature of plants through changes in the composition of genetic material, namely DNA and chromosomes. Changes in the composition of genetic material give rise to new genetic diversity so that the characters displayed are diverse (Lozada *et al*., 2021). New genetic diversity is a hope for a breeder to find new superior characters as an effort to improve character. Mutation induction is done chemically and physically by using mutagens (substances that cause mutations). Examples of chemical mutagens are nitrosomethyl urea and nitrosoguanidine, while examples of physical mutagens are alpha, beta and gamma rays (Yong *et al*., 2021).

Physical mutation induction using gamma-ray mutagens is often used in an attempt to generate new genetic diversity. Gamma rays have a higher penetrating power to plant cells than other mutagens, thereby increasing the possibility of changes in genetic makeup (Verweij *et al*., 2020). Gamma ray mutation induction causes the mutated cells to be charged with high kinetic energy. High kinetic energy causes changes in the composition of DNA and plant chromosomes (Kim *et al*., 2021).

Genetic diversity in mutants (mutated plants) can be observed visually through the appearance or phenotype of plants. Phenotype is a character expressed by living things as a result of the interaction between genotype factors and the environment. Genotype is a trait of genetic composition that is inherited from one generation to the next (Barker *et al*., 2019). Diverse genotype characters can be identified from plant phenotypes through qualitative and quantitative character observations (Sahid *et al*., 2022). Quantitative characters are characters that can be counted such as height, number of leaves, and fruit weight. While, qualitative characters cannot be counted such as fruit shape, leaf shape, and growth type (Álvarez-Carretero *et al*., 2019).

The formation of quantitative characters is strongly influenced by genetic and environmental factors. Quantitative characters are influenced by polygenic genes with complex inheritance of characters and the influence of each gene is very minimal (Dewi *et al*., 2019) Meanwhile, qualitative characters are controlled by major genes or only one to two genes so that the characters displayed are more stable (Lukmanasari *et al*., 2020). The existence of differences in the phenotype of qualitative characters in a plant population is due to genetic diversity and these characters can be passed on to the next generation even with diverse environments (Kao *et al*., 2021). Qualitative characters are different from quantitative characters, so when planted in different environments, they can display different characters from the previous generation because these characters are very sensitive to environmental changes. Therefore, plant breeding efforts through the characterization of the qualitative character of Prentul Kediri cayenne pepper are very necessary for the selection of mutant strain genotypes and as a breeding evaluation material. Selection of mutant genotypes requires data on genetic diversity and relationships between mutant genotypes.

Based on the diversity of qualitative characters in the M0 generation, (Sukmawati *et al*., 2019) showed that gamma ray induction with doses of 100 Gy, 200 Gy, and 300 Gy was able to cause new diversity in the qualitative character of plant habitus. Mutant plants that experienced changes in character in the early generations were caused by physical damage (putative mutants). Putative mutants cannot be called true mutants because they are not yet stable so that it is still possible for character changes to occur in the next generation (van Harten, 1998). The purpose of this study was to determine the phenotype diversity and level of similarity of the qualitative character of the M1 generation of Prentul Kediri cayenne pepper.

2. MATERIALS AND METHODS

Research have been conducted from August 2021 until February 2022. The experiment was implemented in the research area of Ketindan Village, Lawang District, Malang City, East Java Province. Latitude and Longitude coordinates of the location was 7° 50'4" S; 112°40'34" E.

2.1. Research Materials

Cayenne pepper seeds of Prentul Kediri variety with control treatment and M1 seeds was produced by M0 mutation at a dose of 100 Gy, 200 Gy and 300 Gy. Other research materials were polybags with a size of 30 cm 30 cm. Planting media composed of garden soil, cocopeat and compost with a ratio of 1: 1: 1.

2.2. Research Procedure

Cayenne pepper seeds were germinated on the germination tray for 30 days after the appearance of 4 to 6 leaves. The chili seeds were planted in polybags with a distance between polybags of 50 cm 30 cm in open land with an area of 13 m 6 m. A total of 120 plants were planted with details of 15 control plants (P), 45 prentul mutant plants with a dose of 100 Gy (MP1), 45 prentul mutant plants with a dose of 200 Gy (MP2), and 15 prentul mutant plants with a dose of 300 Gy (MP3).

The experimental design used was a non-factorial randomized block design (RBD) with three replications. Each replication consisted of 5 control plants, 15 MP1 plants, 15 MP2 plants and 5 MP3 plants. Observation of quantitative characters consisted of plant height, number of leaves, age of flowering, age of harvest, total number of fruit planted, total fruit weight, fruit length and fruit diameter. The qualitative characters observed consisted of habitus, leaf shape, fruit shape, calyx, and flower position according to the (Mau *et al.*, 2017) guidelines.

2.3. Data Analysis

Data analysis used cluster or similarity analysis. This analysis was performed using the NTSYSpc.2.02i program. According to (Vasilenko et al., 2021), the similarity level analysis produces a dendogram.

3. RESULTS AND DISCUSSION

3.1. Plant Character

The results of the study (Table 1) showed that the diversity occurred in the character of habitus, leaf shape, and fruit shape. Meanwhile, the characters that remain uniform are the appearance of the calyx (base of the fruit) and the position of the flower. The highest percentage of the number of habitus characters in each dose of gamma ray mutation in the M1 population was the upright type, followed by the intermediate type (slightly broad) and the compact type (Table 1). This habitus change to intermediate and compact is very interesting (Figure 1). The grouping of plant growth habitus according to (Ghorbani & Harighi, 2018) are broad, compact, and upright. Intermediate and compact types receive more light because they have a wider canopy than upright types. The more upright the habitus, the lower the light intensity received.

The type of leaf shape with the highest percentage of each dose of gamma ray mutation in the M1 population was the oval type, then the deltoid type (rounded) and the lowest was the lancet type (Table 1). According to (Ghorbani & Harighi, 2018), cayenne pepper plants generally have an oval leaf shape (deltoid) and a lancet leaf shape which is characterized by pointed and slender leaf tips. The type of M1 leaf shape can be seen in Figure 2.

The variety of fruit shape types found were tube, moderately triangular, narrowly triangular, cordate (heart), rectangular (square) and irregular (Figure 3). The type with the highest percentage of the number of mutations in each dose of the M1 population gamma rays was the moderately triangular type and the lowest was the cordate type (Table 1). All control plants had moderately triangular fruit shape. According to (Bulu *et al*., 2020), the varied and unique forms of cayenne pepper can be used to determine the characteristics and quality of chilies desired by breeders.

Table 1. Diversity of qualitative character of cayenne pepper

Populasi	Habitus	(%)	Leaf shape	(%)	Fruit shape	(%)
P (Control)	Dense Intermediate Sparse	100	Deltoid Oval Lancet	100	Tube Moderately triangular Narrowly triangular Heart (Cordate) Rectangular Irregular	0 0 100 0 0 0
MP1 (100 Gy)	Dense Intermediate sparse	55.5 31.1 13.3	Deltoid Oval Lancet	17.8 66.7 15.6	Tube Moderately triangular Narrowly triangular Heart (Cordate) Rectangular Irregular	8.9 8.9 57.8 2.2 6.7 15.6
MP2 (200 Gy)	Dense Intermediate sparse	44.4 33.3 13.3	Deltoid Oval Lancet	22.2 60 17.8	Tube Moderately triangular Narrowly triangular Heart (Cordate) Rectangular Irregular	2.2 6.7 80 4.4 0 6.7
MP3 (300 Gy)	Dense Intermediate sparse	53.3 40 6.7	Deltoid Oval Lanceolate	26.7 60 13.3	Tube Moderately triangular Narrowly triangular Heart (Cordate) Rectangular Irregular	0 20 26.7 0 6.7 46.7

Figure 1. Habitus types: (a) upright, (b) intermediate (slightly broad), and (c) compact

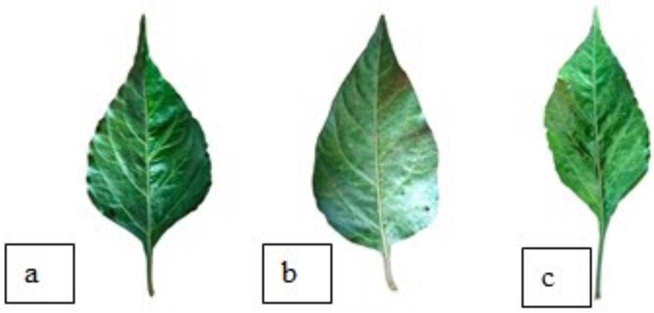

Figure 2. Leaf types: (a) deltoid, (b) oval, and (c) lancet

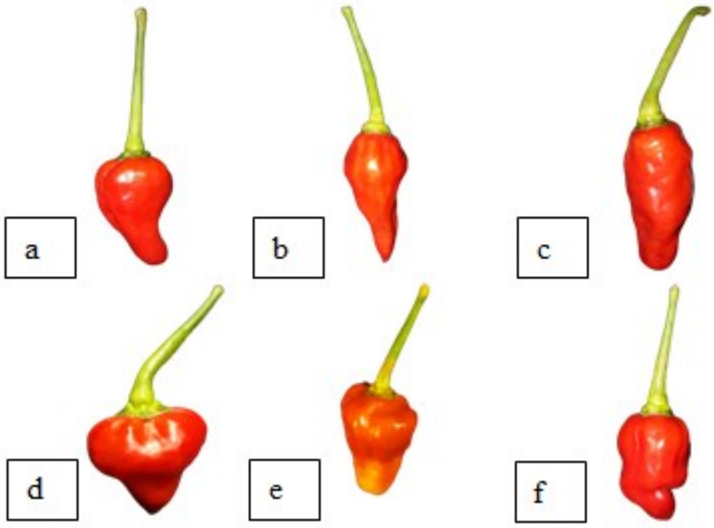

Figure 3. Fruit shape of cayenne peppers: (a) moderately triangular, (b) narrowly triangular, (c) tube, (d) heart (cordate), (e) rectangular, and (f) irregular

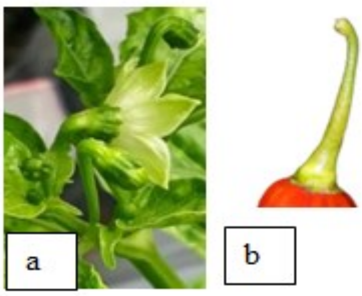

Figure 4. Flower position and calix: (a) upright flower and (b) non enveloping cali

Different levels of sensitivity in each plant cell cause not all cells to be exposed to mutagens so that mutations occur randomly (Schoen & Schultz, 2019). This is likely to occur in plant cells that affect the character of flower position and calyx (base of fruit) which are not affected by mutagens so that they do not change (Figure 4). The flower position on all control and M1 plants was upright. Meanwhile, the shape of the fruit calyx in all plants is non-enveloping. According to (Wang *et al*., 2019) the uniform character of the fruit calyx and flower position can be caused by the occurrence of diplontic selection events where mutant cells cannot compete with normal cells. This causes the plant to grow normally like the initial plant.

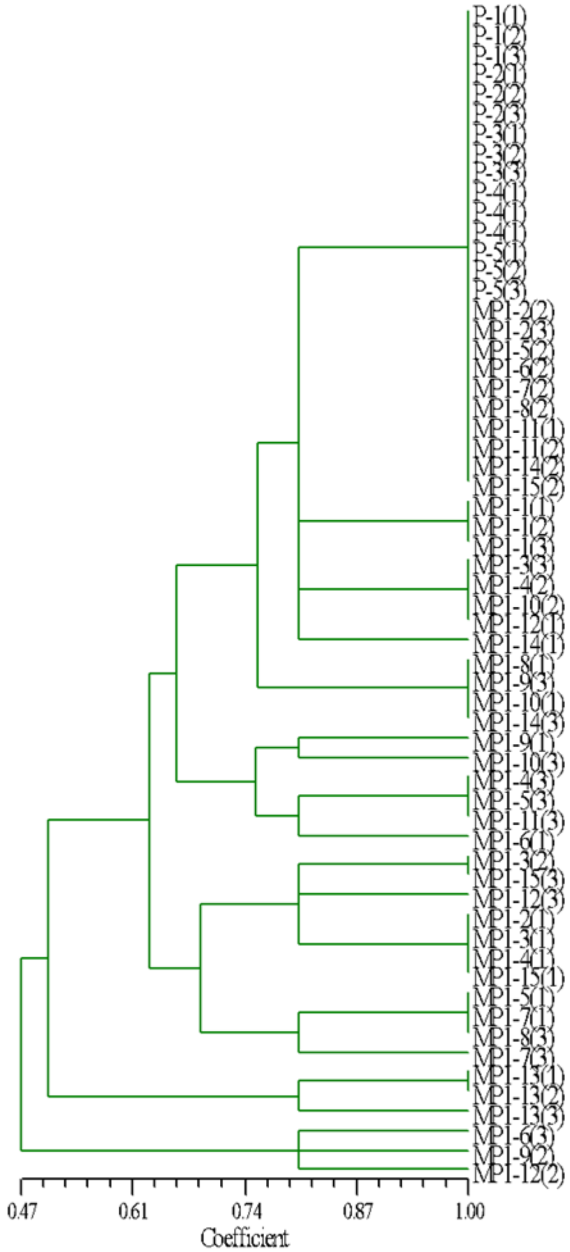

Figure 5. Dendogram similarity for Mutan (M1) with irradiation of 100 Gy

3.2. Similarity or Cluster Analysis

Similarity analysis aims to group all data so that they are clustered in relatively homogeneous character groups (Friston *et al*., 2019). In this case, qualitative characters are clustered into binary data in the form of scoring according to established criteria. The smaller the cluster, the higher the characters similarity of the plants in the cluster. The similarity analysis in the mutant genotype population resulted from mutations of 100 Gy gamma ray irradiation with control plants (Figure 5) show that the range of similarity coefficients is 0.47 – 1. This means that if the coefficient of similarity between genotypes is getting closer to 0.47 (47%) then the level of similarity is getting further away. Conversely, if the coefficient of similarity between genotypes is close to or even 1 (100%) then the level of similarity is getting closer. The level of similarity of mutants that reached 100% was found in clusters C, E, F, H, J, L, N, O, and P. Cluster A consisted of MP1-6(3), MP1-a9(2), and MP1-12(2) mutants with a similarity level of 0.80. Cluster A has the furthest coefficient level of 47% with other individuals. Cluster M consisted of MP1-14(1) mutants which had a similarity coefficient of 0.80 with clusters N, O, and P. Cluster I consisted of MP1-6(1) mutants having a similarity coefficient of 0.75 with clusters J and K. Cluster D consists of mutants MP1-7(3) having a similarity coefficient of 0.80 with cluster E. Cluster B consists of mutants MP1-13(3) having a similarity coefficient of 0.80 with cluster C. The dendogram shows that the resulting mutant population is still there were individuals that were exactly the same as the control plants (P), namely in the P cluster.

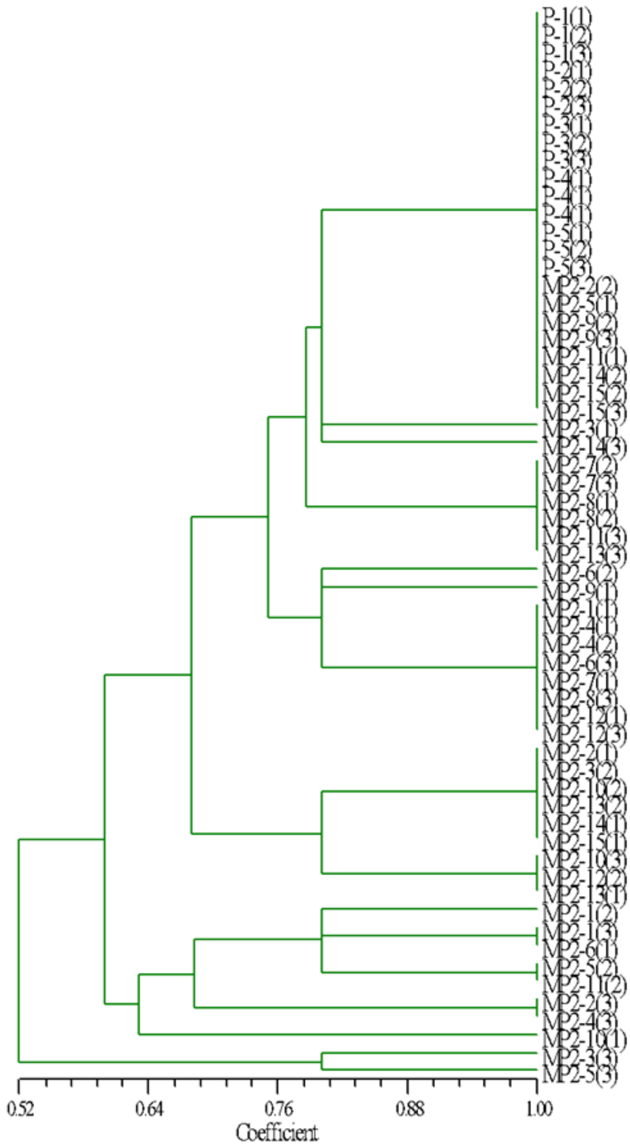

Figure 6. Dendogram similarity for Mutan (M1) with irradiation of 200 Gy

Similarity analysis on the population of mutant genotypes produced by gamma ray irradiation of 200 Gy with control plants (Figure 6) shows that the range of similarity coefficients is in the number 0.52 – 1. The level of similarity of mutants that reaches 100% is found in clusters C, D, E, G, H, I, K, and M. Cluster A consisted of MP2-3(3) and MP2-5(3) mutants with a similarity level of 0.802. Cluster A has the furthest coefficient level of 52% with other individuals. Cluster F consists of the MP2-1(2) mutant having a similarity coefficient of 0.68 with cluster C. Cluster B consists of the MP2-10(1) mutant having a similarity coefficient of 0.63 with clusters C, D, and E. The dendogram also shows that the resulting mutant population still contained characters that were 100% similar to control plants (P), namely in cluster M.

Analysis of the level of similarity in the population of mutant genotypes resulting from mutations of 300 Gy gamma irradiation with control plants (Figure 7) shows that the range of similarity coefficients is in the number 0.59 - 1. The level of similarity of mutants that reaches 100% is found in clusters C, D, G, and I. Cluster A consisted of MP3-2(1), MP3-2(2), and MP3-2(3) mutants with a similarity level of 0.7. Cluster A has the furthest coefficient level of 59% with other individuals. Cluster F consists of the MP3-5(1) mutant which has a similarity coefficient of 0.77 with clusters G, H, and I. Cluster E consists of the MP3-3(2) mutant which has a similarity coefficient of 0.801 with cluster D. In this dendogram, it can be seen that at a dose of 300 Gy the resulting mutant population had characters that were not completely similar to or different from the control plants (P). According to Suwarno et al. (2013) the higher the gamma ray mutation dose will bring out a higher diversity of characters than the lower gamma ray dose.

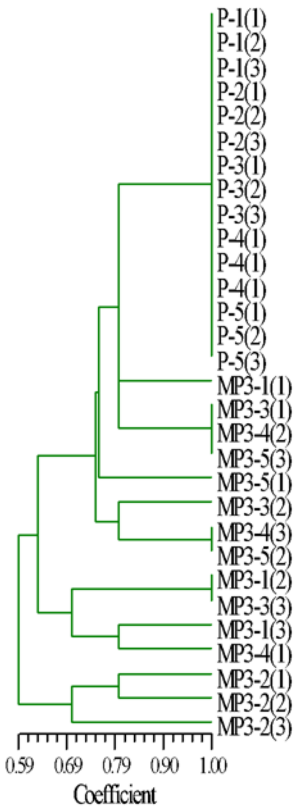

Figure 7. Dendogram similarity for Mutan (M1) with irradiation of 300 Gy

Observations on dendograms show that individuals belonging to the same species with the same dose of gamma-ray mutation do not necessarily have the same degree of similarity. This happens because of the differences in each plant cell in responding to gamma ray irradiation, giving rise to various new characters. According to (Levine & Schwarzbach, 2021) similarity analysis that provides information based on the phenotype or physical appearance of plants still requires information on the level of similarity based on DNA properties. This is because genotypes that have similarities are often found in plants whose phenotypes look different.

4. CONCLUSION

Based on the results of research conducted on 120 plants, it was found that gamma ray mutations in M1 Cayenne Pepper Prentul Kediri caused phenotype diversity in the qualitative character of plant habitus, leaf shape and fruit shape. Qualitative characters that remained uniform in the mutant population (M1) were the calyx character of the fruit and flower position. The level of similarity between individual mutants at each dose of gamma ray mutation is varied. In the mutant population (M1) mutation results from gamma rays at doses of 100 Gy and 200 Gy still found individuals with characters that were 100% similar to control plants. Meanwhile, in the mutant population (M1), the results of mutations in gamma rays at a dose of 300 Gy have not found individuals with characters that are 100% similar to control plants.

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