Similarity Among 10 Cayenne Pepper (*Capsicum frutescens* L.) Mutant Accessions Resulted from Gamma-Ray Irradiation

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ABSTRACT

Cayenne pepper (Capsicum frutescens L.) is a horticultural crop which production is not comparable to the level of public consumption. Chili plants induced by gamma irradiation experienced character changes, which caused the population of mutant accessions to have a diversity of characters. The similarity test of mutant accessions was carried out to determine the relationship distance between accessions. The research was carried out at the Faculty of Agriculture, UPN "Veteran" East Java, Surabaya City, with a geographical location at 7º 9' - 7º 21' South Latitude and at 112º 36' - 112º 54' East Longitude. The experimental design used was a Completely Randomized Design, with 3 replications of each, also using one factor, namely mutant (M_2) or genotype resulting from gamma ray irradiation mutations of cayenne pepper. Heritability tests were also carried out in this study. The results of the heritability test showed that the characteristics of plant height, fruit length, fruit diameter, fruit weight per plant, and number of fruit per plant had high heritability values, namely above 30%. The result of similarity test visualized by the dendogram shows that 10 mutant accessions and the parental have a similarity coefficient ranging at 21% to 78%. The lowest similarity coefficient is owned by M2 accession, specifically at 21%.

1. INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.) is one of the Indonesia's leading commodities. *Capsicum* sp. is a horticultural plant that can be cultivated in tropical areas with a temperate climate (Gris *et al*., 2021). Cayenne pepper production in Indonesia has decreased, namely in 2021 by 8.09% or decrease for 2.5 million tons from 2020 (BPS, 2021). The decline in the production of cayenne pepper in Indonesia is inversely proportional to the increased in consumption demand.

The production of superior varieties is one of the efforts to increase production so that it can meet consumption demand (Djayadiningrat *et al*., 2010). High genetic diversity is an aspect that can influence the success of developing superior varieties (Terryana *et al*., 2020). High genetic diversity causes the potential for certain characters to emerge more widely so that the selection process can be carried out more freely.

Irradiation mutation is a breeding method that has high potential in producing plants with new characteristics. Irradiation mutations can cause genetic changes in plant morphology and physiology. Genetic diversity due to irradiation mutations in morphology can include shape, color, and size. Meanwhile, physiological changes that can occur are resistance to pests, disease, and environmental stress (Damayanti, 2021).

Genotypes resulting from gamma irradiation mutations have the opportunity to become superior parents in crosses. The high diversity that occurs in plant populations resulting from mutations can cause large genetic distances between

genotypes. The long genetic distance between the two parents of a cross breeding is an opportunity for plant breeders to increase variability in morphology and quality (Srinivas & Thomas, 2018), as well as obtain offspring that are superior to the two parents. The similarity test is a method for determining the genetic distance between accessions, so the accession's potential to be used as parents for cross breed can be identified. Cross breed parents that have a high genetic distance or a low similarity index can increase the potential to produce diverse and superior offspring.

The diversity that forms in a population is influenced by genetic and environmental factors. The heritability test is carried out to determine the factors that have the greatest influence on changes that occur in the population, including environmental or genetic factors. Research by Madhu, *et al*. (2023) showed that almost all the characters observed in several chili genotypes had strong GCV (Genetic Coefficient of Variance), strong PCV (Phenotypic Coefficient of Variance), and high heritability. So it can be concluded that the chili genotypes tested have the following characters that are more influenced by genetic factors. The heritability value can also be used to determine the ability to pass on one's character. The research results of Arumingtyas, *et al*. (2023) regarding mutant chilies also showed that significant differences in heritability rates between characters indicate the large number of genetic changes that occurred in the mutant genotype relative to its parents.

This research aims to determine the genetic distance between accessions by testing the similarity of several quantitative characters of mutant cayenne pepper. This research also calculated the heritability values of the ten mutant accessions and their parents, so that the genetic and environmental factors that influence character changes in the population are known.

2. RESEARCH MATERIALS AND METHODS

The research was carried out from February to May 2023 at the Faculty of Agriculture, UPN "Veteran" East Java, Surabaya City, with a geographical location at 7º 9' - 7º 21' South Latitude and at 112º 36' - 112º 54' East Longitude. The study employed a Completely Randomized Design (CRD) with a single factor, consisting of Tulungagung local variety (parent) and 10 accessions (M1 to M10) of 2nd generation mutant cayenne peppers derived from the Tulungagung local variety. These accessions were obtained through gamma irradiation-induced mutations at radiation doses ranging from 100 to 300 Gy, with three replications for each treatment.

Figure 1. Polybag trial to evaluate cayenne pepper similarity of 10 mutant accessions

2.1. Preparation and Cultivation

The research began with seed preparation. Seed preparation was carried out by soaking the seeds in Antracol 70 WP fungicide solution to prevent contamination by unwanted organisms. Pot trials using polybag was conducted as seen din Figure 1. Seed sowing, transplanting, maintenance were carried out which included watering, fertilizing, replanting, and controlling pests, as well as harvesting at 95 days after planting.

2.2. Observation Parameters

The parameters included quantitative characteristics of cayenne pepper plants, namely plant height, number of leaves, flowering age, fruit length (3-5 fruit samples per plant), fruit diameter (3-5 fruit samples per plant), weight per fruit (3-5 fruit samples per plant), fruit weight per plant, and number of fruit per plant. Observations were carried out on 10 mutant accessions of the Tulungagung local variety of cayenne pepper resulting from second generation gamma ray irradiation and parents.

2.3. Data Analysis

2.3.1. Environmental Variance (EV), Phenotypic Variance (PV), Genotypic Variance (GV)

Data obtainesd from observation were subjected to analysis of variance and heritability. Phenotypic Variance (PV), Genotypic Variance (GV), and Environmental Variance (EV) were estimated from the mean squares obtained through analysis of variance, with the formula (Singh & Chaudary, 1979):

$$GV = \sigma^2 g = \frac{MSg - MSe}{r} \qquad (1)$$

$$EV = \sigma^2 e = MSe \qquad (2)$$

$$PV = \sigma^2 p = \sigma^2 g + \sigma^2 e \qquad (3)$$

where MSg is mean square of genotype, MSe is mean square of error, r is replication, and $\sigma^2 g$ is genetic variation.

2.3.2. Genetic coefficient of variance and heritability

Data obtained from observations were subjected to analysis of variance and heritability. Genetic variation was estimated from phenotypic variation minus environmental variation, with the formula:

$$\sigma^2 g = \sigma^2 p - \sigma^2 e \qquad (4)$$

Genetic coefficient of variance (GCV) was used to estimate the extent of genetic diversity for each character, and was estimated using Equation (5) (Singh & Chaudhary, 1979), with μ is avarage of population.

$$GCV = \frac{\sqrt{\sigma^2 g}}{\mu} \text{ x } 100\% \qquad (5)$$

The heritability in broad sense ($h^2{}_{bs}$) was estimated by calculating genetic and phenotypic variance, as the following:

$$h^2{}_{bs} = \left(\frac{\sigma^2 g}{\sigma^2 p}\right) \text{ x } 100\% \qquad (6)$$

According to Moedjiono & Mejaya (1994), the GCV is divided into four categories, including low (<25%), quite low (25% < x < 50%), quite high (50% < x < 75%), and high (<75%). Meanwhile, the estimated heritability value in a broad sense can be categorized as high (>50%), medium (20-50%), and low (≤20%) (Syukur et al., 2015).

2.3.3. Similarity test

The data obtained from the observations was then scored. Measuring the level of kinship and creating a dendogram for binomial data from the observed accessions used the NTSYS 2.0 program (Numerical Taxonomy and Multivariate Analysis Systems). Cluster analysis or grouping was carried out according to Rohlf (1998) using the SAHN method (Sequential Agglomerative Hierarchycal Nested Cluster Analysis).

3. RESULTS AND DISCUSSION

Gamma-ray induction is one of many ways to increase genetic diversity. Research by Nugroho, *et al.* (2021) showed that gamma-ray irradiation mutations at a dose of 450 Gy could increase genetic diversity in quantitative characters, including canopy width and harvest age. Quantitative characters are controlled by the environment and genes, which are controlled by many genes (Syukur *et al.*, 2015). Therefore, in determining diversity in a population, it is necessary to estimate the heritability value, to determine the role of genetic factors in the diversity that occurs.

Tabel 1. Values of EV, PC, GV, GCV, and heritability of M2 population quantitative characters*

	EV	PV	GV	GCV (%)	Heritability (%)
Plant Height	19.58	57.17	37.58	8.54	65.74
Number of Leaves	58.05	73.69	15.63	3.52	21.21
Flowering Age	5.96	6.71	0.75	1.98	11.16
Fruit Length	0.12	0.25	0.13	11.51	53.20
Fruit Diameter	0.01	0.02	0.0079	7.21	38.54
Weight per Fruit	0.04	0.05	0.0068	5.54	14.08
Fruit Weight per Plant	161.41	313.9	152.49	34.99	48.58
Number of Fruit per Plant	55.78	238.1	182.32	44.78	76.57

Note: EV = Environmental Variance, PV = Phenotypic Variance, GV = Genotypic Variance, GCV = Genotypic Coefficient of Variance.

The second generation of mutant population (M_2) is the best generation to identify the mutant plants. M_2 generation has a character that possibly occurred from M_1 segregation to create homozygotes recessive and/or dominant alleles (Page & Grossniklaus, 2002). This research uses control plant populations and M_2 mutants to estimate the value of environmental variation and phenotypic variation, so that the value of genetic variation can be known for estimating broad-based heritability and genetic diversity coefficients. Table 1 shows the values of environmental diversity (EV), phenotypic diversity (PV), genetic diversity (GV), genetic diversity coefficient (GCV), and heritability obtained from observations of quantitative characters.

The results of data analysis show that the GCV value in the population ranges not more than 50%. Quite low GCV (25 to 50%) appears in the characteristics of fruit weight per plant and number of fruit per plant. Meanwhile, low GCV (below 25%) is found in the characteristics of plant height, number of leaves, flowering age, fruit length, fruit diameter, and weight per fruit. This low and quite low GCV value indicates that the quantitative characters observed have fairly uniform characters or narrow genetic diversity. These results indicate that the diversity of characters that emerged in the M_2 mutant cayenne pepper population tested was more influenced by environmental factors. A similar case was also observed in the research results of Chowdhury *et al.* (2023) regarding the genetic analysis of anthracnose resistant and heat tolerant chili inbreed lines based on morpho-physiological characteristics. The research obtained results that the low genotypic coefficients of variance in the population found in stem diameter, total number of branches, days to first maturation, days to first flowering, and photosynthesis rate. It indicated that the environment has a quite much influence on their phenotypic expression.

Heritability is a genetic parameter used in plant breeding activities to determine the extent of the ability of genotypes in a population to pass on their characters on to the next generation (Gumelar, R. *et al.*, 2023). The heritability value estimated from observations is a heritability value in a broad sense that can explain the magnitude of genetic or environmental influence on the phenotypic characters that emerge, as well as influence the success of selection (Hermanto *et al.*, 2017). The heritability value of the analysis results ranges from 11.16% to 76.57%. Where high heritability values (above 50%) appear in the characters of plant height, fruit length, and number of fruit per plant. Moderate heritability values (20-50%) are owned by the characters number of leaves, fruit diameter, and fruit weight per plant. Meanwhile, the flowering age and weight per fruit characters have low heritability values (less than 20%). The results of the heritability estimation show that the plant height, fruit length, and number of fruit per plant are characters that have high heritability. It is suspected that characters with high heritability estimates have genetic factors that are more influential than environmental factors, so that the chance of these characters being passed on to their offspring is higher. This is in line with the research that conducted by Farwah, *et al.* (2020) regarding genetic variability,

heritability, and genetic advance studies in chilli genotypes. The research obtained results that the traits of fruit weight per plant and seed weight per plant exhibited high heritability values, so that these characters have high selection accuracy and genetic gain potential.

Based on the analysis results, the result of genetic diversity and heritability coefficient values do not always in line. This contradicts several research results regarding estimates of heritability and genetic diversity coefficients that have been carried out. Research conducted by Sufiyan (2024) has demonstrated that the traits exhibiting high genetic variability often correspond with high heritability coefficients, indicating the potential for effective selection in breeding programs. The research results shows that yield per plot displays high phenotypic and genotypic coefficients of variation, along with high heritability and genetic advance. However, inconsistent values of genetic diversity and heritability coefficients also occurred in the research results of Naegele, et al. (2016). The research identified variations of peppers for mature fruit phenotypic traits, correlations among fruit traits, and resistance to *Phytophthora* fruit rot. The study found significant differences within all fruit phenotype categories among pepper lines, with broad-sense heritability for fruit traits ranging from 0.56 (shoulder height) to 0.98 (pericarp thickness). This results indicate that certain traits are primarily controlled by genetic factors, others may be more affected by environmental influences, leading to inconsistencies between genetic diversity and heritabilty coefficients. Which indicates that these traits can be improved through trait-based selection.

The kinship test produces a visualization in the form of a dendogram containing the similarity coefficient. The higher the similarity coefficient value shown between accessions, the higher the level of similarity. Analysis of genetic diversity, especially in mutant plants, is something that needs to be done to determine the extent of genetic changes from the original variety. This is in line with the statement of Sumilah et al. (2019), namely, genetic diversity analysis is useful in obtaining information about differences in mutant characters from the original variety and the changes that occur. Apart from that, kinship testing is also useful for determining good crossbreed parents in the next generation. The greater the distance between the parents of the cross, the higher the chance of getting superior offspring. Accessions that are closely related tend not to produce diverse or superior offspring (Lestari et al. 2021).

Figure 1 shows the results of dendogram analysis with similarity coefficients ranging from 0.21 (21%) to 0.78 (78%). There are 4 clusters formed from 30% intercept line on the dendogram. Cluster I includes parents (K) and M5 accession;
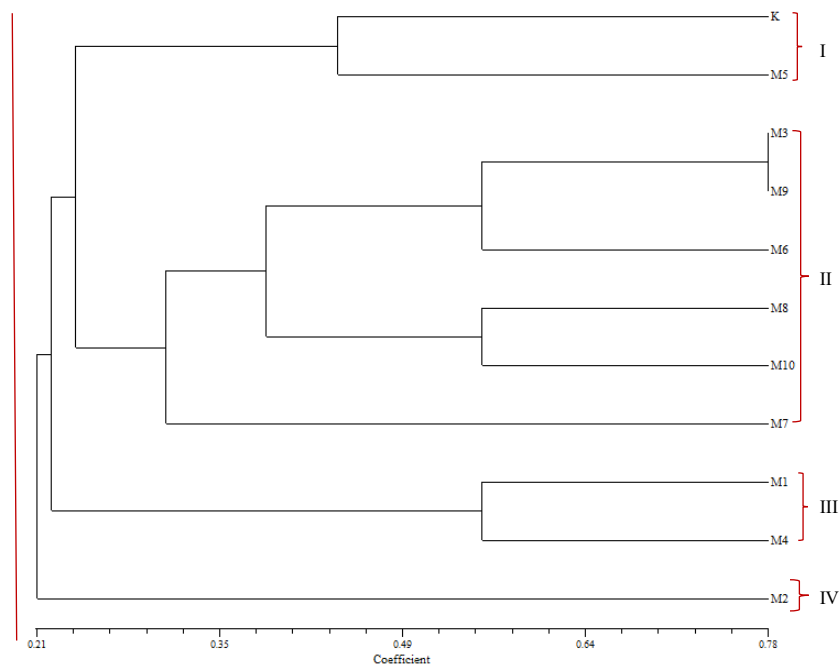

Figure 1. Dendogram of similarity between mutant accessions

cluster II includes M3, M9, M6, M8, M10, and M7; cluster III includes M1 and M4; and cluster IV includes only M2. The closest relationship distance was shown in M3 and M9 accession with a similarity coefficient of 78%. Meanwhile, M2 accession has the furthest relationship distance from other mutant accessions, with similarity coefficient of only 21%. Accessions that are distantly related have great potential to be used as parents for cross breeding because of the superiority of different morphological characters, resulting in superior and diverse offspring (Susilowati & Syukur, 2022). Birchler *et al*. (2010) stated that long kinship distances can increase the occurrence of heterosis (changes in the character and productivity of hybrid plants compared to the average parent), so that they have the potential to produce superior offspring. This is also supported by the statement of Ujianto *et al*. (2012) that crosses with parents who are distantly related are expected to produce offspring with high heterosis values, or have superiority over the average of the two parents.

4. CONCLUSION

Based on the analysis that has been carried out, this research concludes that the M2 accession has the greatest similarity to the parents and other accessions (21%). Furthermore, characters that have high heritability values include plant height, fruit length, and number of fruit per plant with heritability values above 50%. However, the GCV values found in the population shows quite low numbers, so it can be assumed that the characters that appear in the second generation cayenne pepper genotypes are more influenced by environmental conditions or have almost the same characters between the genotypes.

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