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Segregation patterns and transgressive segregation for agronomic characters in red chili (*Capsicum annuum* L.) Laris Variety of M3 generation after gamma-irradiation

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Abstract. The information on genetic parameters need to be known in relation to the selection process for breeding of high yielding plant genotypes. This study aims to estimate the frequency distribution, segregation pattern, and transgressive segregation for agronomic characters in M3 generation of red chili "Laris" variety after gamma-ray irradiation. The research was conducted at the Integrated Field Laboratory, Faculty of Agriculture, University of Lampung from September 2018 to May 2019. This research used single plant method. Data were analyzed using the chi-square test at the 1% level of significance for normal distribution fit and segregation pattern analysis. Transgressive segregation can be seen through the frequency distribution of M_3 generation plants compared to M_0. Results of this study indicated that the characters of dichotomous height, flowering age, harvesting age, plant height, branching level, number of fruits per plant, and fruit weight per plant had a normal distribution and associated with quantitative characters. The number of primary branches had a non-normal distribution and termed as a qualitative character. The segregation pattern of the number of primary branches follows a 15:1 ratio which indicates that the controlling gene is regulated by two genes that work in a double dominant epistatic manner. Transgressive segregation which has a positive value was found in the characters of dichotomous height, number of fruits per plant, and fruit weight per plant.

1. Introduction

Red chili (*Capsicum annuum* L.) is an important vegetable that has high economic value and is cultivated commercially in the tropics, including Indonesia [1]. National chili production in 2017 reached 1,206,272 tons with a harvested area of 142,547 ha, while in 2016 its production was 1,045,591 tons with a harvested area of 123,404 ha. This increase in chili production was attributed to an increase in chili harvested area by 15.37%. In 2017 the national chili productivity reached 8.46 t/ha while in 2016 the national productivity reached 8.47 t/ha [2]. This indicates that the increase in harvested area has not yielded the expected results because productivity is not much different from the previous year. According to [3] the productivity of red chilies in Indonesia is far below its potential productivity which may reach 22 t/ha. The low productivity of chili can be caused by many factors such as the lack of use of superior quality seeds, environmental influences, pest and disease attacks, as well as suboptimal cultivation practices and improper postharvest techniques. One way to overcome this problem is to use superior seeds assembled by plant breeders.

Plant breeding products are cultivars with special or desired characteristics such as high yield, tolerant to marginal conditions (acid soil, drought, high temperature, shading, etc.), resistant to pests and diseases, etc. Assembling such cultivars can be done by selecting various populations. According to, one way of increasing population diversity can be done by inducing physical mutations with gamma-ray irradiation. Previous research by Siahaan S., et al (2018) [4] on chili plants showed that giving gamma irradiation have the potential to produce plants with high production. Theoretically, the mutations that occur will be a random which leads to various possibilities. In irradiated seeds, the chance of mutation is greater in the generations of self-pollinated offspring from irradiated seeds, namely in the M1 and M2 generations. In these generations, segregation has occurred at loci that have undergone mutations so the chance for the appearance of new characters will be higher [5]. In addition, the traits combination possessed by new offspring will appear in the next generation [6]. With the existence of new traits that appear, it allows breeders to select plants with the desired traits or characters.

Selection of the desired character will be easy if it is regulated by a few genes. If more than one gene is involved, the selection period will be longer [7]. Transgressive segregation can be used to select characters that are classified as quantitative characters. Transgressive segregation is gene segregation on quantitative characters that have a distribution range that exceeds the distribution range of their parents. Therefore, it is necessary to look at the number of genes that regulate a plant character by looking at its segregation pattern. According to [8], the segregation level and broad-range recombination are described by the distribution of genotype frequencies. This can be used as an estimate of inheritance patterns and the number of genes involved in controlling a character. This study aims to estimate the frequency distribution, segregation patterns, and transgressive segregation of the agronomic characters of the red chili variety Laris generation M3 resulting from gamma-ray irradiation.

2. Materials and methods

This research was conducted at the Integrated Field Laboratory of the Faculty of Agriculture, University of Lampung at an altitude of 126 masl from September 2018 to March 2019. The chili seeds of the Laris variety used were initially treated with gamma-ray irradiation with a dose of 400 Gy at Center of Research and Development of Isotope Technology and Radiation), BATAN, Pasar Jumat, Jakarta on June 15, 2016. The materials used were pesticide (a.i. carbofuron 3%), fungicide (a.i. mankozeb 80%), insecticide (a.i. profenos), the fly trap with active ingredient methyl eugenol, NPK Mutiara fertilizer, manures, foliar fertilizer, and water. As many as 104 M2-112 generation chili seeds and 40 M0 chili seeds were planted in an experimental plot measuring 3.8 m x 12 m. The unit of experiment was a single plant method. The observed variables were dichotomous height, number of primary branches, flowering age, harvesting age, plant height, branching level, number of fruits per plant, and fruit weight per plant.

Data were analyzed using the chi-square test ($\chi 2$) at the 1% significance level for conformity to the normal distribution [9] followed by the test for skewness and the significance of various theoretical ratios[10]. Estimation of transgressive segregation was based on the graph of frequency distributions, if the graph of the distribution of the population M3 exceeds M0 is interpreted as transgressive segregation [11].

The significance test of the suitability of the segregation pattern of each character with the expected type of segregation was tested with $\chi 2$ [10]. Inheritance of genes that control characters that have a concordance ratio between observed values and expectations is considered as the number of genes that control the observed characters. According to Snyder and David (1957) cited by [12] if the controlling gene is simple, then the M3 population will be adjusted according to several ratios depending on the shape of the graph obtained: 1. If the graph of the distribution of the M3 population shows two peaks, the possible ratios that occur are 3:1 (one full dominant gene), 9:7 (two duplicate recessive epistatic genes), 13:3 (two recessive dominant epistatic genes), or 15 :1 (two duplicated dominant genes). 2. If the graph of the distribution of the M3 population shows three peaks, the possible ratios that occur are 1:2:1 (one imperfectly dominant gene), 9:3:4 (two recessive epistatic genes), 9:6:1 (two duplicate genes with cumulative effect) and 12:3:1 (two dominant epistatic genes). 3. If the graph of the distribution of the M3 population shows more than three peaks, the possible ratio that occurs is 9:3:3:1 (two completely dominant genes) or 6:3:3:4 (one pair of completely dominant genes and one pair of dominant genes in part) if one pair of genes is homozygous recessive, one gene pair will be epistatic to the other gene, whereas if both genes are homozygous recessive, the second gene pair will be epistatic to the first gene pair. 4. If the graph of the distribution of the M3 population shows one peak and the distribution is normally distributed, then the character studied is controlled by many genes. The normality test uses the Chi-square test [9]

3. Results and discussion

The results of research on red chilies of the M3 generation resulting from gamma-ray irradiation showed that the frequency distribution of dichotomous height, flowering age, harvesting age, plant height, branching level, number of fruits per plant, and fruit weight per plant were normally distributed, supported by the skewness test results (Table 1 and Table 2). Characters that are normally distributed are thought to be characters that are controlled by many genes so that they are classified as quantitative characters [13]. According to [14], the distribution of quantitative characters in plants that stretches to the left or right indicates the influence of lethal genes, epistasis, gene linkage, and dominance. Quantitative characters are basically controlled by many genes that have a small contribution in the inheritance of a character and are significantly influenced by the environment, so that individual effects cannot be detected by Mendel's method [15].

The results of this study are in line with the research of [16] on chili plants including the characters of flowering age, fruit weight per plant, and number of fruits per plant included in the quantitative characters. In another study, [17] reported that the characters of flowering age and plant height in lowland rice were controlled by many genes. Likewise in the research of [18, 19] on soybean plants showed that the characters of plant height and seed weight per plant were belong to quantitative characters. In the number of primary branches, the χ^2-value is greater than χ^2-table, so the distribution does not follow the normal distribution (Table 1). This shows that the number of primary branches is a qualitative character which is controlled by a few genes. According to [15], qualitative characters are controlled by a few genes and the segregation pattern follows Mendelian ratios or its modification.

The results of the significance test on the character of the number of primary branches follow a segregation ratio of 15:1 with a 5%-10% chance (Table 3). The 15:1 segregation pattern indicates that the gene that regulates the number of primary branches is epistatically dominant, that is, two genes produce the same material and produce the same phenotype [20, 21]. In [18] study on soybean plants, the harvesting age and weight of 100 seeds followed a segregation ratio of 15:1. In the study of [4] in rice plants, the character of the number of grains per panicle follows a segregation ratio of 15:1.

On the quantitative character of chili plants, the M3 population showed positive transgressive segregation as indicated by one plant having a dichotomous height exceeding M0 in the range of 59 cm to 66 cm (Figure 1). Positive transgressive segregation was found in the number of fruits per plant character indicated by 11 plants having a number of fruits exceeding M0 with a range from 76 fruits per plant to 118 fruits per plant (Figure 2). Positive transgressive segregation was also shown by 33 plants having fruit weight per plant exceeding M0 which ranged from 97.88 g to 193.91 g (Figure 3). Likewise with the research of [22] in chili plants, transgressive segregation was found in the dichotomous height and number of fruits. Transgressive segregation in flowering age characters was shown by 30 plants that exceeded M0 in the range from 33 to 40 day after planting (dap) (Figure 4). Transgressive segregation in harvesting characters was shown by two plants that exceeded M0 in the range of 104 to 108 dap (Figure 5). Transgressive segregation in plant height characters was shown by three plants that exceeded M0 with a range from 40 cm to 60 cm (Figure 6). Transgressive segregation at the branching level was shown by four plants that exceeded M0 with a range from 14 to 16 branches (Figure 7).

Table 1. Chi-square test for suitability of the normal distribution of chili agronomic characters.

No	Plant characters	$\chi2$ value	$\chi2$ 0,01	Interpretation
1	Dichotomus height	2.11ns	9.21	Normal distribution
2	Primary branch number	20.05*	9.21	Non-normal distribution
3	Flowering age	8.42ns	9.21	Normal distribution
4	Harvest age	1315ns	13.28	Normal distribution
5	Plant height	3.36ns	15.09	Normal distribution
6	Branching level	6.82ns	13.28	Normal distribution
7	Fruit number per plant	9.00ns	15.09	Normal distribution
8	Fruit weight per plant	3.81ns	15.09	Normal distribution

Note : * = significant at α 0.01, ns = non-significant at α 0.01

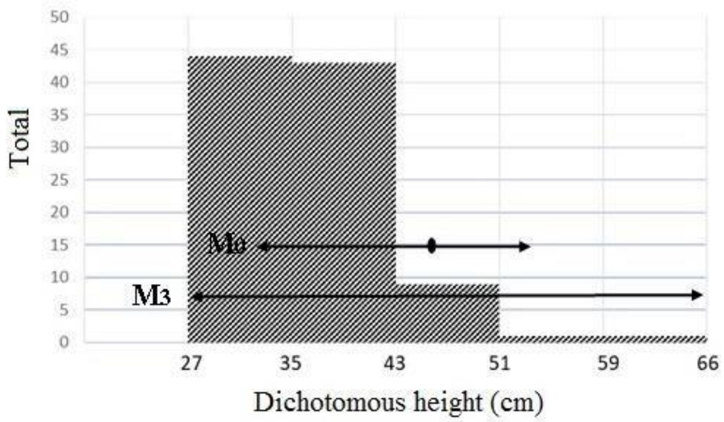
Table 2. Test for skewness of plant characters of M_3 chili generation after gamma-radiation.

No	Plant characters	Value	Interpretation
1	Dichotomous height	0.30	Skewed to the right
2	Flowering age	-0.16	Skewed to the left
3	Harvest age	-0.13	Skewed to the left
4	Plant height	-0.28	Skewed to the left
5	Branching level	0.31	Skewed to the right
6	Fruit number per plant	0.34	Skewed to the right
7	Fruit weight per plant	0.20	Skewed to the right

Table 3. Chi-square test for the suitability of the segregation pattern of the primary branch number of chili

Ratio	Observation (O)	Expectation (E)	x^2value	x^2 0,01	Probability (%)
Two classes					
3:1	96:02	73.5 : 24.5	28.18**		<0.005
9:7	96:02	55.125 : 42.875	69.50**	6.64	<0.005
13:3	96:02	79.625 : 18.375	18.66**		<0.005
15:1	96:02	91.875 : 6.125	3.64^{tn}		0.10 - 0.05
Three classes					
1:2:1	94:02:02	24.5 : 49 : 24.5	262.90**		<0.005
9:3:4	94:02:02	55,125 : 18,375 : 245	62.67**	9.21	<0.005
9:6:1	94:02:02	55,125 : 36.75 : 6,125	63.10**		<0.005
12:3:1	94:02:02	73.5 : 18,375 : 6,125	23.09**		<0.005
Four classes					
9:3:3 : 4 : 1	94:02:01:01	55,125 : 18,375 : 18,375 : 6,125	62.73**	11.35	<0.005
6 : 3 : 3 : 4	94:02:01:01	36.75 : 18,375 : 18,375 : 24.5	142.75**		<0.005

Note : ** = Significant at α 0.01
ns = non-significant at α 0.01



Note: ● = highest number of plant

Figure 1. Histogram of conformity of the normal distribution of chili character on dichotomous height

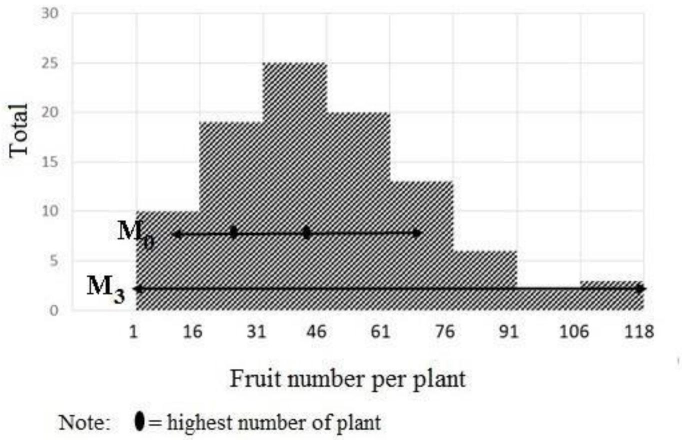


Figure 2. Histogram of conformity of the normal distribution of chili character on fruit number per plant

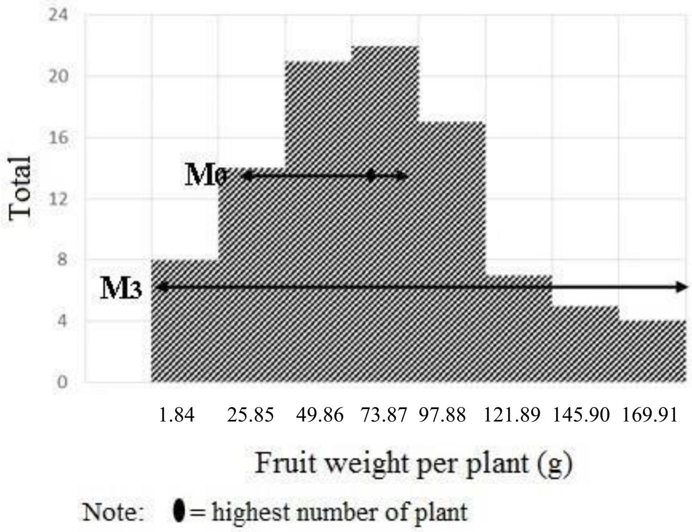


Figure 3. Histogram of conformity of the normal distribution of chili character on fruit weight per plant

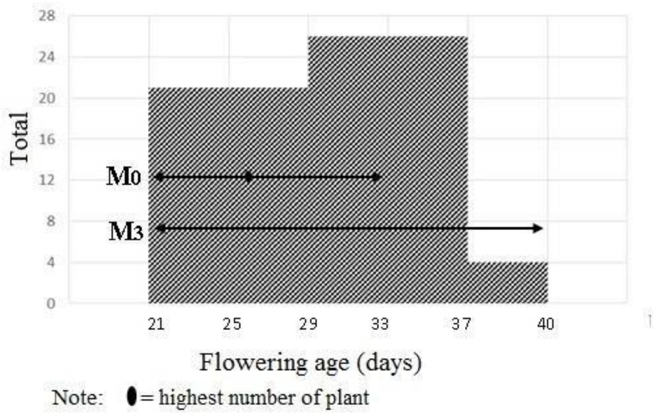


Figure 4. Histogram of conformity of the normal distribution of chili character on flowering age

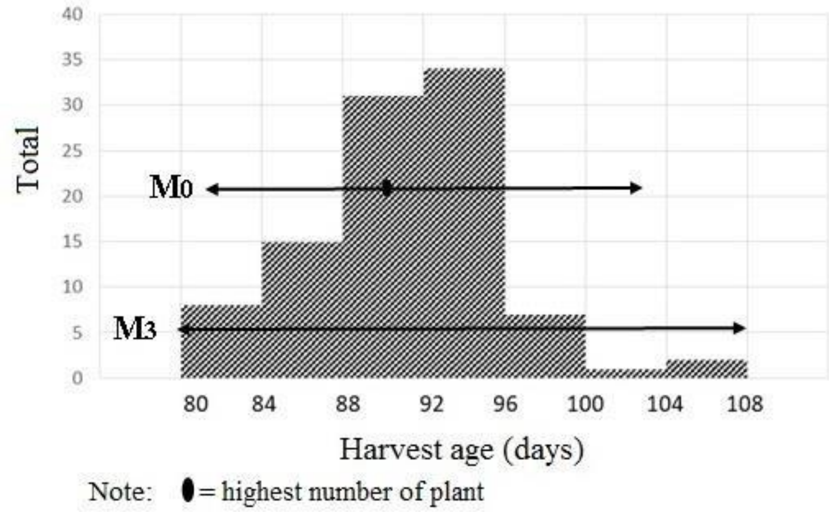


Figure 5. Histogram of conformity of the normal distribution of chili character on harvest age.

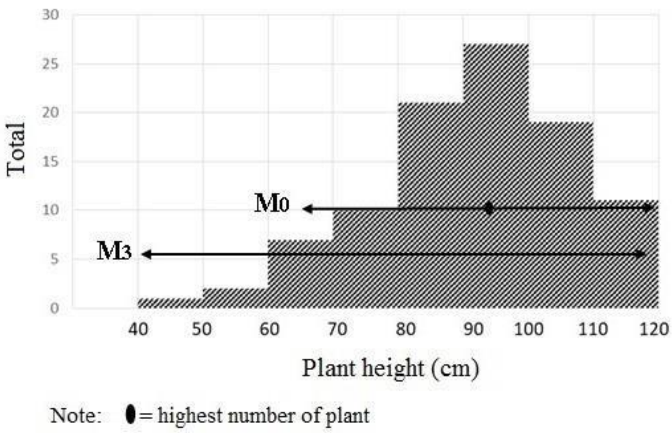


Figure 6. Histogram of conformity of the normal distribution of chili character on plant height.

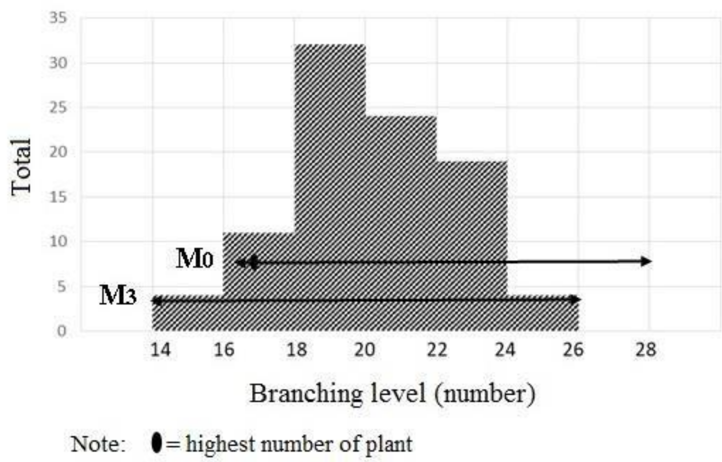


Figure 7. Histogram of conformity of the normal distribution of chili character on branching level

4. Conclusion

The frequency distribution of characters that are normally distributed includes dichotomous height, flowering age, harvesting age, plant height, branching level, number of fruits per plant, and fruit weight planted including quantitative characters. Meanwhile, the character of the number of primary branches has a frequency distribution that is not normal and includes a qualitative character. The character segregation pattern of the number of primary branches follows the segregation ratio of 15:1 and Positive transgressive segregation is indicated by the character of dichotomous height, number of fruits per plant, and fruit weight per plant.

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