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GENOTYPIC SELECTION ON RED CHILI PLANTS RESISTANT TO ANTHRACNOSE DISEASE AT M_2 GENERATION

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ABSTRACT

Genotypic selection on red chili plants resistant to anthracnose disease at M_2 generation. A superior anthracnose resistant cultivar was studied to overcome the low production due to anthracnose in red chili. For the development of superior cultivars, it was necessary to select genotypes that were resistant to anthracnose. Selection effectiveness was determined by wide diversity and high reliability. The purpose of this study was to investigate the diversity and heritability of agronomic characters and resistance to anthracnose on chili plants, and to select genotypes that were resistant to anthracnose. This research was conducted with a design without repetition. The plant material used was the seed of the results of gamma ray mutations in generation M_2. The results of this study were: the broad diversity of phenotypes found in all characters observed, while all characters of the genotype observed had broad criteria except plant height at flowering and harvest, and at seedling period. The genotype that should be planted in the next generation was genotype number 136. Genotype number 136 was very resistant to anthracnose infection.

Key words: anthrax, diversity, disease resistance, heritability, selection

ABSTRAK

Seleksi genotipe tanaman cabai merah tahan penyakit antraknosa pada generasi M_2. Kultivar tahan antraknosa unggul dicari untuk mengatasi produksi rendah karena antrasnosa dalam cabai merah. Untuk pengembangan kultivar unggul, perlu untuk memilih genotipe yang resisten terhadap antraknosa. Keefektifan pemilihan ditentukan oleh keanekaragaman yang luas dan keandalan yang tinggi. Tujuan dari penelitian ini adalah untuk melihat keragaman dan heritabilitas karakter agronomi dan ketahanan terhadap antraknosa pada tanaman cabai, dan untuk memilih genotipe yang resisten terhadap antraknosa. Penelitian ini dilakukan dengan desain tanpa pengulangan. Bahan tanaman yang digunakan adalah benih hasil mutasi sinar gamma pada generasi M2. Hasil penelitian ini adalah: keragaman luas fenotip yang ditemukan pada semua karakter yang diamati, sedangkan semua karakter genotipe yang diamati memiliki kriteria luas kecuali tinggi tanaman saat berbunga dan panen, dan pada periode pembibitan. Genotipe yang harus ditanam pada generasi berikutnya adalah nomor genotipe 136. Nomor genotipe 136 sangat tahan terhadap infeksi antraknosa.

Kata kunci: antraks, heritabilitas, keanekaragaman, resistensi penyakit, seleksi

INTRODUCTION

Chili productivity in 2016 was 8.47 tons/ha. Production of 8.47 tons/ha was still relatively low (Badan Pusat Statistik, 2017). One of the causes of low chili production was lack high-quality seeds and high pest and disease infections. Pest and disease infections were the most dominant factors in reducing chili productivity in Indonesia (Hakim *et al*., 2014).

One of the important diseases in chili production in the tropics and humid regions is anthracnose. Anthracnose is a disease caused by fungus *Colletotrichum* spp. This pathogen can infect before and after plant harvest and reduce production up to 50% or more (Nurhayati, 2007; Syukur *et al*., 2009). The use of superior varieties resistant to anthracnose is an effort to overcome the problem of anthrax infection. Therefore, it is necessary to develop superior cultivars resistant to anthracnose.

The development of superior cultivars is began with the selection from the diverse existing sources. Selection of effectiveness is determined by the diversity width. One source of diversity could be obtained by mutation. Mutations are change in genetic material.

Mutation was one method that had been shown to increase genetic diversity which could be applied to support plant breeding programs (Yunita *et al*., 2014).

Mutation induction mostly used gamma irradiation because gamma rays irradiation had a higher frequency of mutation results and was easier to apply (Gaswanto *et al*., 2016), gamma rays had high energy, on crossing plant material could cause changes in the tissue itself, cells, structure or composition of genetic material (genomes, chromosomes, genes, DNA). Changes in the genetic traits of these plants went to the direction of better or worse (Soeranto, 2003). In addition to the wide diversity, the effectiveness of selection was determined by the estimated high heritability. The estimated value of high heritability meant that genetic factors had more role than environmental factors.

Gaswanto *et al*. (2015) conducted a mutation study using gamma rays to obtain chili which was categorized as resistant and rather resistant to *Begomovirus* infection. Manzila *et al*. (2015) reported that chili mutations using *ethyl methanesulfonate* (EMS) to produce mutant chili lines that were resistant to tolerance to *chili venal mottle virus* (ChiVMV).

This study was initiated by mutating chili seeds in August 2016. The doses used were 0 Gy (without irradiation), 100 Gy, 200 Gy, 300 Gy, and 400 Gy. Mutation results were planted in October 2016 until March 2017. The results obtained, there were differences in the appearance of each dose. The higher dose of gamma rays, the shorter the seeds that grew. This showed that the influence of mutations on the growth of chili seedlings (Sa'diyah, 2016). At the dose of 300 Gy the seed weight was the highest (Sa'diyah *et al*., 2017). Therefore, what continued on the next planting was the seed of generation M_2 from the dose of 300 Gy.

In this study, it was expected that chili plants that were irradiated with gamma rays experienced genetic changes in a better, lowered direction and could lead to a wide diversity. Therefore, the purpose of this study was to understand whether the changes in nature occurred or not, judging with the estimated value of heritability. In addition, it was also seen whether diversity occurred in generation M_2. The best genotype selection was based on diversity, the estimated value of high heritability, resistance to anthracnose disease and the large number of fruits.

MATERIALS AND METHODS

Research Site. This study was conducted in the integrated field laboratory of the Faculty of Agriculture, University of Lampung from June to December 2017. Gamma radiation had been carried out at the Research and Development Center for Isotopes and Radiation Technology, Pasar Jumat, Jakarta in August 2016.

Planting material used in this study were 84 generation M_2 chili seeds (second generation mutations) from the Ferosa variety given a dose of 300 Gy. Plants produced from the seeds of generation M_2 each had differences due to gamma ray mutations. Therefore, observation and inoculation was carried out on each plant.

Inoculation was done by spraying a suspension of spores *of Colletotrichum* spp. at a density of 10^6 spore/mL at flowers and early fruiting stages. The suspension of the *Colletotrichum* spp. spore was made by means of *Colletotrichum* spp. isolated from anthracnose-like infected chili. The anthracnose-chili peppers were cut in size of 2 mm^2 and then incubated on PDA media, then purified by being transferred to the next PDA media until pure spores of *Colletotrichum* spp were harvested from one week old isolates. The spores then were suspended in 30 mL of distilled water.

Variables observed were: anthracnose disease resistance, seed height, number of primary branches, plant height at flowering, plant height at harvest, flowering age, number of flowers, harvest age, incubation period, and number of fruits. Resistance to anthracnose was measured based on the incidence of disease. Scores and criteria for resistance to anthrax were measured using Yoon's modified method of Syukur *et al*. (2007) (Table 1). The occurrence of disease or disease incidence (DI) was calculated using the formula:

$$DI = \left(\frac{n}{N}\right) \times 100\%$$

DI = disease incidence
n = number of infected fruits
N = total fruit number

Analyses of the range of phenotypes(σ_f^2), environmental variance(σ_e^2), and genetic variance (σ_g^2) were calculated as:

$$\sigma_p^2 = \frac{\sum_{i=I}^{n} (Xi - \mu)^2}{N} \qquad \sigma\frac{2}{e} = \sigma_P^2 \mathrm{M}_0$$

$$\sigma_g^2 = \sigma_p^2 - \sigma_e^2$$

σ_g^2 = variability of genotypes
Xi = value of plant observations to -i
μ = mean population value
N = number of plants observed
σ_p^2 = variability of phenotypes
σ_e^2 = variability of environments

The variability of phenotypes observed in the parent population was the same as the environment because the population of parents was genetically uniform and the variability of genotypes was zero. The variability of environmental of the parental population was as the same as that of the offspring since the parental and their offspring populations were planted in the same environment. The standard deviation calculation formula ($\sqrt{\sigma^2}$) is:

$$\sqrt{\sigma^2} = \sqrt{\frac{\sum_{i=I}^{n}(Xi-\mu)^2}{N}}$$

$\sqrt{\sigma^2}$ = standard deviation
Xi = observation value to -i
μ = mean population value
N = number of plants observed.

Estimating heritability in the broadest sense (H) used the formula:

$$H = \frac{\sigma_g^2}{\sigma_f^2}$$

H = broad sense heritability
σ_g^2 = variability of genotypes
σ_f^2 = variability of phenotypes.

Remark: High heritability (H > 0.5); Moderate heritability (0.2 < H < 0.5); Low heritability (H < 0.2) (Mendez-Natera *et al*., 2012).

The best genotype criteria were based on wide diversity, high predictability of heritability and great resistance to anthracnose.

RESULTS AND DISCUSSION

The Diversity of Phenotypes and Genotypes. The diversity of phenotypes for the characteristics of seedling height, number of primary branches, flowering age, number of flowers, plant height at flowering, age of harvest, plant height, incubation period, and number of M_2 generation chili characterized as broad criteria (Table 2). The same results were obtained by Syukur *et al*. (2010) where the characters of plant height, flowering age, harvest age, and the number of chili plants had a wide diversity of phenotypic values.

Almost all variables had wide genotype diversity except the characters of plant height at flowering, plant height at harvest, and the incubation period (Table 2). Narrow genotype diversity on the characters of plant height at flowering and plant height at harvest was similar with the research conducted by Nura (2015). In the Nura's study (2015) it was shown that plant height character had a narrow diversity of genotypes. The narrow range of genotypes explained that plants were genetically uniform. Plants that were genetically uniform could cause the selection process to the next generation to be less effective (Aryana, 2012). This meant that there was no need to do a selection based on characters that had a narrow diversity of genotypes.

In general, a character that had a wide variability of genotypes would have a wide variability of phenotypes as well. However, in this study there were characters who had narrow genetic diversity but had a wide variability of phenotypes. The wide variability of phenotypes showed that there were differences in appearance on these characters, because the expressions varied in the same genotype.

Values of Estimated Heritability. Value of heritability for resistance character, seed height and number of primary branches classed in the high criteria. The character of the number of fruits was classed in the criteria of moderate. The estimated heritabilities classed in the low criteria were the character of flowering age, number of flowers, plant height at flowering, harvest age, crop height at harvest, and incubation period (Table 3). There were differences in criteria between this study and the research conducted by Satriawan *et al*. (2017). Satriawan *et al*. (2017) conducted a study on F_2 generation chili plants found that the estimated value of heritability was influenced by the character of the population, the evaluated genotype sample, calculation

Table 1. Chili resistance scores and criteria for anthracnose based on disease incidence

Score	Incidence (%)	Criteria
1	0–10	Highly Resistant
2	11–20	Resistant
3	21–40	Moderate Resistant
4	41–70	Susceptible
5	> 70	Highly Susceptible

method, flexibility of genotype evaluation, linkage imbalance, and experimentation.

The high predictive value of heritability in a character indicated that the phenotype produced by the character was determined by genetic factors rather than environmental factors. In contrast, low heritability showed that the appearance produced was determined by environmental factors rather than genetic factors. Moderate heritability showed that the character phenotype was influenced by genetic factors and environmental factors of equal size (Satriawan *et al*., 2017; Syukur *et al*. (2011). Some characters had a heritability value of 0.00, which was the characters of flowering plants, crop height and the incubation period, similar to that reported by Maryenti *et al*. (2015) who obtained a heritability value of 0.00. This occurred because the genotype diversity of the character was negative. The diversity that had a genotype value that was negative was considered zero (Hallauer & Miranda, 1988), so the value of the heritability would also be equal to zero.

Resistance to Anthrax Disease. Resilience to anthracnose in chili was predicted based on the incidence of the disease. Disease events were used to determine the score and criteria for resistance to anthracnose in chili (Syukur *et al*., 2007). Disease was observed from the fruit with symptoms on green fruit and red fruit after inoculation during the flowering phase and fruiting phase.

Table 2. Variability criteria for phenotype and genotype of M_2 generation mutant chili

Character	σ_f^2	Criteria	σ_g^2	Criteria for
Seedling height	46.22	Wide	43.99	Wide
Number of prima branches	52.37	Wide	35.62	Wide
Age of flowering	1609.95	Wide	214.16	Wide
Number of flower	192,642.06	Wide	9625.58	Wide
Plant height at flowering	516,34	Wide	0	Narrow
Age of harvest	10911.18	Wide	2013.16	Wide
Plant height at harvest	2502.44	Wide	-1100.43	Narrow
Incubation Period	135.40	Wide	0	Narrow
Severity of disease	207.50	Wide	149.09	Wide
Number of fruits	6215.91	Wide	1502.61	Wide

Diversity criteria based on Anderson and Banchrof in Wahdah (1996) were $\sigma_f^2 > 2\sigma_f^2$: wide diversity and $\sigma_f^2 < 2\sigma_f^2$: narrow diversity. $\sigma_g^2 > 2\sigma_g^2$: wide diversity and $\sigma_g^2 < 2\sigma_g^2$: narrow diversity.

Table 3. Heritability in the broad sense of generation M_2 mutant chili

Character	H	Criteria
Seed	0.95	High
Number of primary branching	0.68	High
Age of flowering	0.13	Low
Number of flower	0.05	Low
Plant height at flowering	0,00	Low
Age of harvest	0,18	Low
Plant height at harvest	0,00	Low
Incubation period	0,00	Low
Severity of disease	0,75	High
Number of fruit	0,24	Moderate

High heritability (H> 0,5); Moderate heritability (0.2 <H <0.5); Low heritability (H <0.2) (Mendez-Natera, 2012).

The lowest disease resistance score for anthracnose in generation M_2 was found in genotypes number 136 and 93 (Table 4), indicating that genotypes number 136 and 93 had resistance to anthracnose disease. Genotype number 136 was infected by anthrax at the time of the red fruit harvest with a percentage of 2.38% disease incidence, and on genotype 93 the percentage of disease incidence was 8.70%. There were several genotypes where 0% infection on red fruit could not be classed in low category because several genotypes with a percentage of 0% in the red fruit harvest produced a high percentage of infection when the fruits were green. This was because the incubation period of the disease on each genotype was different. In addition, many of the 0% infection percentage in red fruit did not have red fruit because fruit had symptoms of the disease when green. The lowest infection during the green harvest was found in genotypes number 94 and 136 with 0% infection percentage, this indicated that the infection did not occur when the fruit was green.

In generation M_2, there were genotypes that had resistance to anthracnose, namely genotypes 136 and 93 (Table 4), while generation M_0 or without mutation (Table 5) all tested plants were very susceptible to anthracnose disease. This showed an increase in resistance to chili anthracnose disease due to 300 Gy gamma ray mutations. This increased resistance to anthrax was a positive expression of mutating genes.

Selection of the Best Genotypes. Phenotype was an observable characteristic of an organism that was regulated by genotype and environment and the interaction between genotypes and the environment. Genotype was an individual's genetic state. Selection would be effective if a character had a broad genotype diversity criterion and a high predictive value for heritability. In character studies which had a wide variety of genotypes there were characteristics of disease resistance, seedling height, number of primary branches, flowering age, harvest age, number of flowers, and number of fruits. However, the estimated value of heritability with high criteria was only on the character of disease resistance, seedling height and number of primary branches. This meant that the selection would be effective on the character of the resistance to disease of the seedlings and the number of primary branches. The character of the number of fruits had a wide variability of genotypes and the estimated value of heritability was moderate, so the character of the number of fruits could also be considered as a selection criterion.

Based on the resistance to anthracnose, the plants which would be continued in the next generation were plants of genotype 136 and 93. In genotype 136, the number of healthy red chili fruits was 41, infected on one red fruit and without infection on green fruits. In genotype 93, there were 21 healthy red chili fruits, 2 red fruits were infected and no infection on green fruits. However, the number of fruits in genotype 136 was only 42 and genotype 93 only 23 which were very low when compared to genotype 90 with 29 healthy red fruits, 37 infected red fruit, 326 infected green fruits. Genotype 136 had 10 cm seedling height and 4 primary branches, plant number 93 had 10.5 cm seedling height and 4 primary branches, while genotype 90 had 10.5 cm seedling height and 7 primary branches.

CONCLUSION

All observation characters had a wide diversity of phenotypes. Almost all of the characters observed had a wide diversity of genotypes, except for plant height at the beginning of flowering, plant height at harvest, and incubation period which had a narrow diversity of genotypes. High heritability was found in seed height, number of primary branches and severity of disease, while moderate heritability in fruit counts per plant. The selected genotypes based on anthracnose disease resistance were genotypes number 136 and 93.

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Table 4. Criteria for resistance to anthracnose in chili varieties Ferosa generation M_2

No Gen. M2	Healthy red fruit	Infected red fruit	Green fruit infected	Total infected fruit	Fruit numbers	DI	Criteria
136	41	1	0	1	42	2.38	Highly Resistant
93	21		0	2	23	8.70	Highly Resistant
94	13	4	8	12	25	48	Susceptible
127	12	5	8	13	25	52	Susceptible
132	11	0	13	13	24	54,17	Susceptible
63	16	8	13	21	37	56,76	Susceptible
144	12	4	31	35	47	74,47	Highly Suscept
79	12	1	36	37	49	75,51	Highly suscept
76	7	12	12	24	31	77.42	Highly suscept
22	10	8	28	36	46	78.26	Highly suscept
142	11	7	34	41	52	78.85	Highly suscept
115	10	10	28	38	48	79.17	Highly suscept
121	14	1	53	54	68	79, 41	Highly suscept
147	9	0	37	37	46	80.43	Highly suscept
105	3	5	8	13	16	81.25	Highly suscept
35	5	5	18	23	28	82.14	Highly suscept
58	22	5	99	104	126	82,54	Highly suscept
125	5	5	20	25	30	83,33	Highly suscept
131	9	3	43	46	55	83,64	Highly suscept
84	14	9	70	79	93	84,95	Highly suscept
65	5	10	19	29	34	85,29	Highly suscept
61	3	0	19	19	22	86,36	Highly suscept
114	5	1	34	35	40	87.5	Highly suscept
145	6	3	42	45	51	88.24	Highly suscept
82	3	3	20	23	26	88.46	Very susceptible
91	5	0	39	39	44	88,64	Highly suscept
78	4	2	32	34	38	89,47	Highly suscept
28	8	1	75	76	84	90,48	Highly suscept
150	4	1	38	39	43	90,70	Highly suscept
32	10	16	82	98	108	90.74	Highly suscept
68	6	7	53	60	66	90.91	Highly suscept
70	9	11	96	107	116	92.24	Highly suscept
122	2	0	24	24	26	92.31	Highly suscept
108	8	6	92	98	106	92.45	Highly suscept
73	20	50	196	246	266	92.48	Highly suscept
90	29	37	326	363	392	92.60	Highly suscept
37	7	20	69	89	96	92.71	Highly suscept
2	1	0	13	13	14	92.86	Highly suscept
46	4	7	47	54	58	93.10	Highly suscept
43	6	7	77	84	90	93.33	Highly suscept
137	3	0	48	48	51	94.12	Highly suscept
57	11	11	182	193	204	94.61	Highly suscept
51	2	4	32	36	38	94.74	Highly suscept
111	4	1	77	78	82	95.12	Highly suscept

Table 4. Continued

No Gen. M2	Healthy red fruit	Infected red fruit	Green fruit infected	Total infected fruit	Fruit numbers	DI	Criteria
141	4	0	79	79	83	95.18	Highly suscept
34	1	6	14	20	21	95.24	Highly suscept
47	1	2	19	21	22	95.45	Highly suscept
55	2	0	46	46	48	95 , 83	Highly suscept
7	1	2	27	29	30	96.67	Highly suscept
33	2	0	58	58	60	96.67	Highly suscept
15	1	0	30	30	31	96.77	Highly suscept
80	5	1	150	151	156	96.79	Highly suscept
29	4	15	112	127	131	96.95	Highly suscept
81	2	0	66	66	68	97.06	Highly suscept
49	1	3	32	35	36	97.22	Highly suscept
21	5	19	157	176	181	97.24	Highly suscept
48	7	6	243	249	256	97.27	Highly suscept
64	2	2	70	72	74	97.30	Highly suscept
13	3	9	108	117	120	97.5	Highly suscept
92	1	9	35	44	45	97.78	Highly suscept
106	2	0	94	94	96	97.92	Highly suscept
31	1	26	23	49	50	98	Highly suscept
53	1	0	49	49	50	98	Highly suscept
130	1	0	49	49	50	98	Highly suscept
107	1	12	48	60	61	98.36	Highly suscept
71	1	2	60	62	63	98.41	Highly suscept
72	1	0	65	65	66	98.48	Highly suscept
24	2	2	132	134	136	98.53	Highly suscept
39	1	18	58	76	77	98.70	Highly suscept
62	1	0	77	77	78	98.72	Highly suscept
8	1	0	90	90	91	98.90	Highly suscept
26	1	4	87	91	92	98.91	Highly suscept
128	1	3	90	93	94	98.94	Highly suscept
20	1	3	162	165	166	99.40	Highly suscept
3	0	9	39	48	48	100	Highly suscept
6	0	8	150	158	158	100	Highly suscept
11	0	1	103	104	104	100	Highly suscept
12	0	5	252	257	257	100	Highly suscept
16	0	4	46	50	50	100	Highly suscept
45	0	2	89	9 1	91	100	Highly suscept
66	0	7	54	61	61	100	Highly suscept
74	0	2	40	42	42	100	Highly suscept
75	0	2	88	90	90	100	Highly suscept
118	0	12	12	24	24	100	Highly suscept

DI = Disease Incidence. Resilience Criteria = Very Resistant, DI: 0-10 %; Resistant, DI: 11-20 %; Moderate Resistant, DI: 21-40 %; Susceptible, DI: 41-70%; Highly Susceptible, DI:> 70%.

Table 5. Criteria for resistance to anthracnose in the varieties of Ferosa generation M_0

No.plants	Healthy red fruit	Infected red fruit	Infected green fruit	Total infected fruit	Fruit number	DI	Score	Resilience Criteria
9	1	0	24	38	47	80.85	5	Highly suscept
21	8	5	77	22	26	84.62	5	Highly suscept
24	0	4	78	38	44	86.36	5	Highly suscept
18	1	0	31	54	61	88.52	5	Highly suscept
2	0	3	257	82	90	91.11	5	Highly suscept
17	0	1	45	25	27	92.59	5	Highly suscept
16	3	3	69	41	43	95.35	5	Highly suscept
8	4	6	88	94	98	95.92	5	Highly suscept
1	9	7	31	24	25	96	5	Highly suscept
7	0	4	64	72	75	96	5	Highly suscept
4	0	14	32	31	32	96,88	5	Highly suscept
20	0	0	79	34	35	97.14	5	Highly suscept
26	0	0	77	40	41	97.56	5	Highly suscept
22	0	0	42	48	49	97.96	5	Highly suscept
3	2	0	41	82	82	100	5	Highly suscept
5	2	0	25	260	260	100	5	Highly suscept
6	7	2	52	46	46	100	5	Highly suscept
10	0	0	48	68	68	100	5	Highly suscept
11	1	0	34	46	46	100	5	Highly suscept
12	4	0	22	79	79	100	5	Highly suscept
13	1	2	46	77	77	100	5	Highly suscept
14	0	0	22	42	42	100	5	Highly suscept
19	6	0	38	48	48	100	5	Highly suscept
23	0	0	32	22	22	100	5	Highly suscept
25	1	1	39	32	32	100	5	Highly suscept
Average	2	2	56	57.8	60	95.87	5	Highly suscept

DI= Disease Incidence. Resilience Criteria= Very Resistant, DI: 0–10%; Resistant, DI: 11–20%; Moderate Resistant, DI: 21–40%; Susceptible, DI: 41–70%; Highly Susceptible, DI: > 70%.

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