Sterility of 20 F₁ genotypes derived from hybridization of several chilli’s lines with M₁ male sterile

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Abstract. Open pollinated (OP) chili varieties seeds had been irradiated previously by gamma rays (400-600 Gy) to obtain four M₁ sterile plants for this study. These sterile plants could be used as a breeding material for F₁ male sterile hybrid variety. The purpose was to evaluate sterility of 20 F₁ genotypes derived from hybridization of several chilli’s lines with four M₁ sterile plants. The research was conducted at Indonesian Vegetables Research Institute, Lembang (1,250 m above sea level) from April to December 2018. The research used a randomized complete block design (RCBD) with three replications. Population of each evaluated F₁ genotype was 10 plants/replication. Sterility trait evaluation was observed by fruit setting ability of various flower’s grouping positions (lower, middle, upper) on each F₁ genotype. Results show that there was one genotype categorized as sterile, one genotype as partial sterile, thirteen genotypes as partial fertile, and five genotypes as fertile lines. Genotype 04 could be selected as a maintainer, whereas genotype RG-1 as a restorer line. Sterility of partial sterile lines with unstable trait could be increased by isolating and collecting the gene pool using back cross technique between their F₁ progenies with maintainer lines for at least five generations.

1. Introduction

Most chilli’s breeding programs are focused on F₁ hybrid varieties. The use of F₁ hybrid will contribute more advantages in terms of high yield quality-quantity, uniformity performance, and resistance to main pest and disease [1]. F₁ hybrid is defined as a variety that produced seeds by hybridization directly between two or more parent lines and there is a heterosis or heterobeltiosis phenomena between them [2]. However, one obstacle to disseminate F₁ hybrid variety is high price of seed, thus small scale farmers are reluctant to use it. The high price of F₁ hybrid seeds is caused by high production cost to emasculate the flower of a female parent [3]. Generally F₁ hybrid seeds are produced by large scale companies, so they can hire many workers to carry out emasculation.

Efficiency of production cost can be undertaken by using male sterile lines, thus emasculation cost can be eliminated. In chili crop, male sterile lines already had been used on F₁ hybrid seed production for long time [4]. But it is not easy to find male sterile lines because as a recessive trait. Male sterile lines usually are controlled by genetic or cyto-genic. On chili’s sterility was controlled by cyto-genic male sterile (CGMS), it is meaning based on interaction between sterile gene inside and outside of nucleus [5].

F₁ male sterile hybrid is known as three lines breeding method, i.e cyto-genic male sterile (S-rfrf), maintainer (N-rfrf), and restorer (N/S-Rfrf) [6]. The first step in breeding program for F₁ male sterile hybrid variety is to find source of the gene. For that, one of the ways to obtain the male sterile gene by mutation induction through gamma rays irradiation [7]. Mutation is a changing fastly and inherited to
the progenies, thus occur a changing structure and composition in level of genome, chromosome, gene or DNA [8], but they are random and unpredictable [9].

The chili seeds of several open pollinated varieties were irradiated gamma rays (400-600 Gy) and succeeded in isolating four M1 individuals identified as male sterile plants [7]. Then these four M1 individual plants were crossed hybridization with several pure lines such as their parents (M0) or other lines. As a result of the hybridization, 20 genotypes of F1 progenies had to be identified with the sterility rate to determine categories as male sterile, maintainer, and restorer lines.

The research purpose was to determine categories as male sterile, maintainer, and restore lines of 20 F1 chilli genotypes derived from hybridization between four M1 individual male sterile plants with several pure lines.

2. Materials and methods
The research was conducted at Indonesian Vegetables Research Institute (IVEGRI), Lembang (1,250 m asl), from April to December 2018. The evaluated materials were 20 F1 chili genotypes derived from hybridization between four M1 individual male sterile plants with several pure lines. The research used a randomized complete block design with three replications. The population number of each genotype was 10 plants per replication.

The experimental land was prepared by plowing and hoeing, then applied horse manure (doses 30 t ha\(^{-1}\)) with NPK 16: 16: 16 as basic fertilizer. After that the land was covered by silver plastic mulch with a spacing of 40 x 60 cm. The plant maintenance included watering, subsequent fertilization, weeding, crop protection by pesticides twice a week, and harvesting. The observed variables included: (1) Flowering time (DAP); (2) Rate of sterility (%); (3) Fresh weight (g) and anther length (mm); (4) Number and diameter of pollen (µm); (5) Shape and color of pollen; (6) Weight (g), length (cm), and diameter of fruit (mm).

Evaluation rate of sterility was conducted at generative phase by observing fruiting ability of each evaluated genotype. The observations were carried out on 10 flowers in each position group (lower, middle, upper) of plant, thus there was a total of 30 observed flowers per plant. Each observed flower was labeled. The sterility rate (%) was grouped into 4 categories [10] namely: fertile (if fruit setting failure < 20%), partially fertile (if fruit setting failure in range of 20% - 54%), partially sterile (if fruit setting failure in range of 55% - 90%), sterile (if fruit setting failure > 90%).

Pollen viability observation was carried out also to verify sterility trait with the following procedure: pollen viability was done by taking pollen in the morning at anthesis, then stained by acetocarmine 1% substance [11], then after 30 minutes carried out observation under a microscope with 10x magnification. If pollen’s color is red, that indicates viability. Observation of the viable pollen number was done three times in several point views then averaged, be calculated using a formula:

\[
Pollen\ \text{viability (\%)} = \frac{\text{The\ stained\ pollen\ number\ in\ a\ point\ of\ view}}{\text{Total\ observed\ pollen\ in\ a\ point\ of\ view}} \times 100\%
\]

Heterosis estimation was calculated by mid-parent heterosis (MP) which F1 hybrid vigor compared with mid parents performance [12].

3. Results and discussion
3.1. Flowering time and rate of sterility
In Table 1 can be seen that there was no difference significantly in flowering time of all evaluated F1 genotypes. Their flowering time was in range of 55-60 days after sown (DAS). It is caused by all evaluated genotypes include indeterminate type. Hence if there was a difference, it still within tolerance limit of 1-5 days. In general chilli’s indeterminate is slower in flowering time than determinate types, so harvesting period usually also longer [13].
Sterile trait is defined as inability of a plant to set fruit naturally without assisted by another pollen hybridization. The phenomenon is very complex, be influenced by genetics such as disruption of the meiosis process and allele incompatibility in formation of male gametes and can be influenced also by environments such as cytoplasmic conditions, temperature, and plant nutrition [14]. In Table 1 can be seen that average fruit setting percentage of flowers was in range of 5.25-91.40%. It was known that only one F1 genotype as sterile category namely M1-04 x 04. This genotype could be used as a male sterile line for F1 cyto-genic male sterile (CGMS) hybrid variety program.

Genotype 04 as the parent line of M1-04 sterile could be selected as a maintainer line candidate. The maintainer line is used to maintain sterile trait, it’s means is a hybridization between sterile with maintainer line will obtain sterile progenies again. The maintainer line is very important and valuable because it is not easy to find it, therefore seed companies will keep it well [15].

3.2. Fresh weight and anther length
In table 2 can be known that observation fresh weight and length of anther showed that sterile plants were lighter and smaller than fertile. Thus there was a reduction for the variable in sterile plants. This was due to imperfect development of anther wall. The anther wall consisted of epidermis, endothelium, and two layers of membrane and tapetum. In sterile plants occurred tapetum damage caused by cutting off nutrient supply and followed by pressing to the room which filled by a half of mother pollen cell, thus these cells became die [16].

3.3. Number and diameter of pollen
In table 2 can be seen that average pollen number showed a difference significantly between sterile and fertile plant. In sterile plant was only containing 14-15 pollen per anther, but more than 100 pollen in fertile plant. It can be seen also that diameter of pollen in sterile plant (45.2 µm) was smaller than fertile

Table 1. Flowering time and sterility rate of 20 F-1 chili genotypes

| No | F1 genotype                        | Flowering time (DAS) | Percentage of fruit setting failure (%) | Sterility Category* |
|----|------------------------------------|----------------------|----------------------------------------|---------------------|
| 1. | M1-04 x RG 1                       | 58 a                 | 5.25 c                                 | Fertile             |
| 2. | M1-04 x RG 24                      | 56 a                 | 10.90 de                               | Fertile             |
| 3. | M1-12 x RG 14                      | 55 a                 | 16.90 d                                | Fertile             |
| 4. | M1-20 x RG 24                      | 56 a                 | 18.45 d                                | Fertile             |
| 5. | M1-22 x RG 11                      | 59 a                 | 18.86 d                                | Fertile             |
| 6. | M1-20 x RG 11                      | 55 a                 | 20.50 d                                | Partially fertile   |
| 7. | M1-12 x RG 11                      | 57 a                 | 22.50 cd                               | Partially fertile   |
| 8. | M1-22 x RG 24                      | 57 a                 | 25.82 c                                | Partially fertile   |
| 9. | M1-22 x RG 1                       | 58 a                 | 26.67 c                                | Partially fertile   |
| 10. | M1-12 x RG 24                      | 55 a                 | 30.45 c                                | Partially fertile   |
| 11. | M1-12 x RG 1                       | 56 a                 | 40.70 bc                               | Partially fertile   |
| 12. | M1-04 x RG 14                      | 58 a                 | 45.45 b                                | Partially fertile   |
| 13. | M1-22 x RG 14                      | 60 a                 | 50.25 b                                | Partially fertile   |
| 14. | M1-04 x RG 11                      | 57 a                 | 50.20 b                                | Partially fertile   |
| 15. | M1-20 x RG 1                       | 58 a                 | 52.00 b                                | Partially fertile   |
| 16. | M1-20 x RG 14                      | 57 a                 | 52.25 b                                | Partially fertile   |
| 17. | M1-20 x 20                         | 58 a                 | 52.80 b                                | Partially fertile   |
| 18. | M1-22 x 22                         | 59 a                 | 54.00 b                                | Partially fertile   |
| 19. | M1-12 x 12                         | 56 a                 | 86.38 a                                | Partially sterile   |
| 20. | M1-04 x 04                         | 59 a                 | 91.40 a                                | Sterile             |

Note: Mean followed by the same letters on the same columns are not significant according to Duncan’s multiply range test at 0.05 level.
plant (72.4 µm). This result same with [16] which reported that pollen diameter in sterile plant was smaller than normal plants.

Table 2. Average of fresh weight and length anther from male sterile and fertile of F-1 genotypes

| No  | Genotype             | Sterility Category* | Anther Fresh weight (g) | Lenght (cm) | Number | Pollen Diameter (µm) |
|-----|----------------------|---------------------|-------------------------|-------------|--------|----------------------|
| 1.  | M1-04 x 04           | Sterile             | 0.0041 c                | 0.21 a      | 14.15 c | 45.2 b               |
| 2.  | M1-12 x 12           | Partially sterile   | 0.0074 b                | 0.30 a      | 25.35 c | 68.5 a               |
| 3.  | M1-20 x 20           | Partially fertile   | 0.0062 b                | 0.32 a      | 98.50 b | 70.1 a               |
| 4.  | M1-22 x 22           | Partially fertile   | 0.0080 ab               | 0.32 a      | 115.40 b | 62.6 a              |
| 5.  | M1-04 x RG 1         | Fertile             | 0.0095 a                | 0.35 a      | 525.60 a | 72.4 a              |

Note: Mean followed by the same letters on the same columns are not significant according to Duncan’s multiply range test at 0.05 level

3.4. Shape and color of pollen
Staining by acetocarmine (1%) substance under microscope showed that there were two types of shapes and colors of pollen. In general pollen of sterile plant was wrinkled and unstained by acetocarmine 1% substance, whereas fertile plant had pollen with symmetrical round and it’s color was red (figure 1). Result of staining and pollen shape of sterile plants are closely related to microspores damage occurrence after tetrad stage during meiosis process [17][18] which is possibly caused by cutting off nutrient supply. Hence the consequence while stained pollen by acetocarmine substance won’t be colored and become wrinkle due to had no starch content [16].

![Shape and color of pollen](image)

Figure 1. (a) Fertile (left) and sterile (right) of flower; (b) Shape and color of pollen

3.5. Weight, length, and diameter of fruit
In table 1 can be known that there were five F₁ genotypes included fertile category namely M1-04 x RG 1, M1-04 x RG 24, M1-12 x RG 14, M1-20 x RG 24, M1-22 x RG 11. Therefore actually lines of RG 1, RG 24, RG 14, RG 11 can be selected as restorer candidates due to their ability to restore progeny’s fertility. However, it must occur heterosis affect performance of their F₁ hybrid. Usually heterosis effect performance will occur if two parent lines have distinct genetic relationship [19].

Evaluation of weight, length, and diameter of fruit five F₁ fertile genotypes showed that only genotype F₁ (M1-04 x RG 1) weighted one fruit (7.03-7.34 g) was higher than the best parent (5.72 g) performance, while fruit length (11.62-12.01 cm) was higher than average two parents’ lines (10.60 cm) with good uniformity. Then according to diameter and shape of fruit were included large chili with diameter size of more than 1.0 cm and a bumpy skin surface. Thus genotype RG-1 can be selected as a candidate for restorer parent line (N/S RfRf) (figure 2).
Figure 2. Fruit performance of lines of male sterile (M1-04 x 04), restorer (RG1), and F1 hybrid genotype

On sterile partial lines with sterility trait unstable could be increased their heterosis potential to F1 progenies by isolating and collecting the gene pool of male sterile lines [20]. The breeding technique can be used to obtain stable sterile line through back cross between their F1 progenies with maintainer parent lines for at least five generations.

4. Conclusion and suggestion
The research result showed that there was one genotype categorized as sterile, one genotypes as partial sterile, thirteen genotypes as partial fertile, and five genotypes as fertile lines. Genotype 04 could be select as a maintainer, whereas genotype RG-1 as a restorer line. On sterile partial lines with trait unstable could be increased their sterility by isolating and collecting the gene pool by back cross technique between their F1 progenies with maintainer lines for at least five generations

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