Induced mutation by gamma rays on performance of MV3 Callistephus chinensis at lowland

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Abstract. Callistephus chinensis has beautiful flower with high sale price and usually grow at highland/plateau. An experiment was carried out to evaluate the effect of induced mutation by gamma rays on performance of third generation (M3V3) Callistephus chinensis at lowland. Tiller of Callistephus chinensis was induced mutation by gamma rays at 0 Gy (Yo), 5 Gy (Y1), 10 Gy (Y2), 15 Gy (Y3) and 20 Gy (Y4) at first generation (M1V1). Plant from M1V1 was selected based on earlier flowering as plant material for M3V3. There were 12 genotypes selected. Twelve genotype and Yo were planted at lowland (125 m above sea level) in Tembalang - Semarang. The results showed that induced mutation by gamma rays increased height, number of leaves, number of flower, flower stalk length of M3V3 Callistephus chinensis at lowland. Number of tiller were not significantly different between induced mutation plant and control, except for Y1-U4-11 and Y3-U4-10. Colour of flower was not significantly different between induced mutation plant and control, except for some of Y1-U3-10 genotype. Most flower colour was purple, while some of Y1-U4-9 genotype (Y1-U4-9-5) were pink. The conclusion was Callistephus chinensis can adapted in lowland due to induced mutation by gamma rays.

1. Introduction

Callistephus chinensis, the type genus of Asteraceae, contains approximately 152 species widely distributed in Eurasia [1]. Propagation of Callistephus chinensis, is through tiller. Callistephus chinensis is self pollinated species. The plants grow well at 1276 m above sea level (asl) [2]. Colour of flower are pink and purple. Appearance of first floret was at 50 – 60 days after planting and full flowering was at 66 – 80 days after planting [3]. Callistephus chinensis has beautiful flower with high sale price. The flower has varied uses such as cut flower, bedding plants in the garden and potted plants. An effort has to do to fulfill the increasing demand. Efforts that can be done to overcome this are through plant breeding to produce adaptive varieties of Callistephus chinensis in the lowland and early flowering.

The first step of plant breeding is creating plant genetic diversity [4] that can be done through hybridization or induced mutation. Mutation is the change of genetic material that inherited to next generation. Induced mutation is mutation through the exposure of plant propagules to physical and chemical mutagen agents [5]. Physical mutagen that most commonly used are gamma and X-rays [6, 7]. Ethyl methanesulphonate (EMS), diethyl sulphate (DES), methyl nitroso urea (MNH), ethyl nitro urea (ENH), ethyleneimine (EI) are the most commonly used as chemical mutagenic agents [8]. Induced mutation was used widespread in plant breeding programmes that led to officially release more than 2700 mutant plant varieties throughout the world [9]. In Indonesia, some mutant ornamental...
plants have been released such as mini roses (namely Rosanda, Julikara and Rosmarun), chrysanthemum (namely Mustika Kania), cut rose (namely Rosma) [10].

Induced mutation by gamma rays on *Hibiscus rosa-sinensis* showed some morphology changes such as type and colour of flowers, leaves type and plant height. Irradiation of 12.5 Gy, 17.5 Gy and 20 Gy caused yellow-whiteness flower and wave leaves [11]. Induced mutation by gamma rays at 15 – 90 Gy on *Dianthus caryophyllus* produced 19 mutants. The mutant plants were quantitatively different in petal and colour shape. The character mutant plants were mostly recognized in MV2 generation. This character was stable until third generation (MV3) [12]. Mutation breeding on pineapple by gamma rays showed that significantly variation of mutan was noticed in MV3. Some mutant also seemed to be more stable in MV3 generation [13]. Induced mutation in *Celosia cristata* resulted in change of flower colour and shape [14].

Some of mutation breeding only focus on morphology changes such as flower colour and shape. There are not many research done to change plant adaptation to lowland by induced mutation. *Callistephus chinensis* usually grow at highland/plateau. In order to fulfill the increasing demand of *Callistephus chinensis*, the breeding programme has to be done to produce adaptive varieties of *Callistephus chinensis* in the lowland. So, an experiment was carried out to evaluate the effect of induced mutation by gamma rays on performance of third generation (MV3) *Callistephus chinensis* at lowland.

2. Materials and Methods
2.1. Mutagenesis
At first generation (MV1), tiller of *Callistephus chinensis* was irradiated with gamma rays at at 0 Gy (Y0), 5 Gy (Y1), 10 Gy (Y2), 15 Gy (Y3) and 20 Gy (Y4) in Centre for Application of Isotope and Radiation – National Nuclear Energy Agency of Indonesia. Irradiated plant were planted at highland in Bandungan – Semarang Regency (915 asl). Plants from MV1 were selected based on the change of flower colour compared to control treatment (0 Gy). There were 13 genotype that selected from MV1. Those genotype were planted as second generation (MV2) at highland in Bandungan – Semarang Regency (915 asl).

2.2. Plant Material and Location
The material were plants at third generation (MV3). Those plants were selected from MV2 based on earlier flowering. There were 12 genotypes selected (Y1-U1-9, Y1-U4-11, Y1-U5-5, Y1-U5-6, Y2-U1-15, Y2-U1-12, Y2-U4-20, Y2-U5-14, Y3-U1-10, Y3-U5-14, Y3-U5-9, Y4-U5-16) and Y0 (0 Gy) as control. There were seven (7) plants for each replicate. Y1-U1-9 consists of 7 replicates. One replicates for genotype Y1-U1-11. Five replicates for Y1-U5-5, Seven replicates for Y1-U5-6. Four replicates for Y2-U1-15. One replicate for Y2-U3-12. Two replicates for Y2-U4-20. One replicate for Y2-U5-14. Three replicate for Y3-U1-10. Four replicates for Y3-U4-14. Three replicates for Y4-U1-9. One replicate for Y4-U5-16. Two replicates for Y0 as control. Twelve genotype and Y0 were planted at lowland (125 m above sea level) in Tembalang - Semarang. Type of soil was latosol.

2.3. Methods
Experimental design used completely random design. Each replicate used bed of 0.5 m x 3 m. Tiller were planted at 40 cm in bed. The distance between bed were 0.75 m. Total beds were 41 beds. Soil tillage was done before planting. Application of manure was done after soil tillage and one week before planting. Dosage of manure was 10 ton/ha. Anorganic fertilizer were applied according to recommendation dosage [15].

Height, number of leaves, number of tiller, number of flower, flower stalk length and flower colour of all plant were recorded. Height, number of leaf and number of tiller were measured before generative stage (2 month after planting). Number of flower, flower stalk length and flower colour were measured four month after planting. Observation of flower colour refers to Royal Horticulture Colour Chart (RHCC).
2.4. Statistical Analysis

Recorded data were analyzed for diversity with coefficient of variance and followed by a Two – tailed test at 5 % to determine the significance different between control and each genotype. T value for equal variance used pooled variance :

\[
T_{\text{value}} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2} \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}}
\]

T value for unequal variance used separated variance as follows :

\[
T_{\text{value}} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2 + s_2^2}{\frac{n_1}{s_1^2} + \frac{n_2}{s_2^2}}}}
\]

\[
\bar{X}_1 = \text{Average of control treatment (0 Gy)}
\]

\[
\bar{X}_2 = \text{Average of genotype}
\]

\[
n_1 = \text{Plant number of control treatment (0 Gy)}
\]

\[
n_2 = \text{Plant number of genotype}
\]

\[
s_1^2 = \text{Variance of control treatment (0 Gy)}
\]

\[
s_2^2 = \text{Variance of genotype}
\]

3. Results and Discussion

3.1. Plant Growth

T-test showed significant different on plant height and number of leaves of *Callistephus chinensis* compared to control (0 gy). Irradiation of gamma rays were not affected number of tiller at third generation of *Callistephus chinensis* at lowland, except for genotype Y1-U4-11 and Y3-U3-10 (Table 1).

Table 1. Plant height (cm), number of leaves and number of tiller of *Callistephus chinensis* at third generation (MV3)

| Genotype     | Plant Height | Number of Leaves | Number of Tiller |
|--------------|--------------|------------------|------------------|
| Yo (control) | 17.9 ± 3.5   | 14.1 ± 3.3       | 4.6 ± 2.1        |
| Y1-U4-9      | 51.1 ± 17.7* | 75.6 ± 37.4*     | 4.0 ± 2.3m       |
| Y1-U4-11     | 40.0 ± 21.2* | 70.6 ± 20.6*     | 7.4 ± 2.7*       |
| Y1-U4-5      | 54.5 ± 12.8* | 85.4 ± 30.3*     | 5.4 ± 2.8m       |
| Y1-U4-6      | 46.9 ± 18.0* | 69.1 ± 36.9*     | 4.3 ± 2.9m       |
| Y2-U1-15     | 47.7 ± 16.1* | 78.2 ± 36.3*     | 4.5 ± 3.7m       |
| Y2-U1-12     | 39.5 ± 11.1* | 55.8 ± 23.7*     | 7.3 ± 4.6m       |
| Y2-U1-20     | 32.6 ± 16.0* | 53.9 ± 27.9*     | 7.1 ± 6.3m       |
| Y2-U3-14     | 36.4 ± 11.7* | 59.4 ± 20.3*     | 4.9 ± 3.0m       |
| Y3-U3-10     | 47.8 ± 17.3* | 67.8 ± 28.9*     | 2.7 ± 1.8*       |
| Y3-U3-14     | 29.6 ± 17.4* | 45.8 ± 31.8*     | 6.6 ± 5.6m       |
| Y4-U3-10     | 57.1 ± 10.2* | 90.5 ± 28.8*     | 4.2 ± 3.2m       |
| Y4-U3-16     | 64.9 ± 7.40* | 84.9 ± 14.5*     | 3.9 ± 2.2m       |

*Significantly different with control (0 Gy) based on T-tailed test at 5%*

Twelve genotype of *Callistephus chinensis* at third generation (MV3) had higher plant height compared to control plant (Figure 1). Irradiation gamma rays affected plant height of *Callistephus chinensis* at lowland. The highest plant height was observed at Y4-U3-16 (64.9 cm). The lowest plant height was recorded at genotype Y3-U4-14 (29.6 cm). Increasing plant height because of gamma rays irradiation (75 Gy) also was observed at third generation (MV3) of *Dianthus caryophyllus* [12]. Plant
height of *Callistephus chinensis* at this experiment was lower than *Callistephus chinensis* cultivar Phule Ganesh Purple (93.75 cm) and cultivar Phule Ganesh Violet (102.25 cm) [16]. This difference of plant height because of the effect of gamma irradiation and also experiment location. *Callistephus chinensis* cultivar Phule Ganesh Purple and cultivar Phule Ganesh Violet were planted without gamma rays’ radiation. Those plants were grown at mid hills condition of Himachal Pradesh India (1275 asl). Coefficient of variance (CV) of genotype $Y_1-U_5-9$, $Y_1-U_4-11$, $Y_1-U_5-6$, $Y_2-U_1-15$, $Y_2-U_3-12$, $Y_2-U_5-20$, $Y_2-U_3-14$, $Y_3-U_3-10$, $Y_3-U_4-14$ were more than 25%. Coefficient of variance is more than 25% indicated that there were very diverse of plant height among plant in the same genotype. Plant height of genotype $Y_1-U_5-5$, $Y_4-U_3-9$, $Y_4-U_5-16$ and control were not very diverse (CV < 25%). Plant height variation due to genotypes has also been reported [17].

![Figure 1](image1.png)

**Figure 1.** a. Plant height of control plant b. Plant height of genotype $Y_3-U_4-14$ c. Plant height of genotype of $Y_1-U_4-9$

Number of leaves of all genotypes *Callistephus chinensis* at MV$_3$ was significantly higher than control plant. Number of leaves were in the range of 45 – 90 leaves. Number of leaves of *Callistephus chinensis* at this research was higher than *Callistephus chinensis* cv Kamini (30 – 43 leaves) [18]. Irradiation of gamma rays increased significantly number of leaves of *Callistephus chinensis* at MV$_3$. Irradiation of gamma rays is the most popular among the physical mutagens because of the use convenience and their capability to infiltrate deep into plant material such as seed or tiller. Gamma rays generate substitutions of nucleotide and delete 2 – 16 bp [8]. Coefficient of variance of genotype $Y_4-U_5-16$ and control plant were less than 25% that indicated those genotypes were not very diverse. Genotype $Y_4-U_5-16$ also had plant height that was not very diverse (Figure 2). Number of tiller of ten genotype *Callistephus chinensis* at MV$_3$ were not significantly different with control plant. Genotype $Y_1-U_2-11$ had number of tiller significantly higher than control plant, while genotype $Y_2-U_3-14$ had number of tiller less than control plant.

![Figure 2](image2.png)

**Figure 2.** a. Genotype $Y_3-U_3-16$ (uniform in plant height, number of leaf, and flower stalk length). b. Genotype $Y_1-U_4-9$ (diverse in plant height, number of leaf, number of tiller and number of flower)
3.2. Floral attributes
Number of flower and main stalk length of flower of all genotype Callistephus chinensis at MV3 were significantly different than plant control (0 Gy) (Table 2). Irradiation of gamma rays affected plant height and main stalk length of flower of Callistephus chinensis at MV3 that was grown in lowland. Flower colour of Callistephus chinensis at MV3 was purple (2.5 P 8/6, 2.5 P 10) and pink (2.5 RP 6/8) 10).

Table 2. Number of flower each plant, Flower stalk length (cm) and flower colour of Callistephus chinensis at third generation (MV3).

| Genotype | Number of flower | Flower stalk length | Flower colour |
|----------|------------------|---------------------|---------------|
| Yo (control) | 13.6 ± 3.2 | 10.8 ± 4.5 | 2.5P 8/6 |
| Y1-U4-9 | 81.9 ± 58.4* | 45.8 ± 13.4* | 2.5P 8/6 and 2.5RP 6/8 |
| Y1-U4-11 | 86.6 ± 27.9* | 54.6 ± 7.6* | 2.5P 8/6 |
| Y1-U5-5 | 78.6 ± 50.8* | 52.4 ± 11.1* | 2.5P 8/6 |
| Y1-U5-6 | 69.4 ± 44.3* | 43.3 ± 12.0* | 2.5P 8/6 and 2.5 P 10/5 |
| Y2-U1-15 | 52.9 ± 29.3* | 46.5 ± 8.7* | 2.5P 8/6 and 2.5 P 10/5 |
| Y2-U3-12 | 35.3 ± 6.3* | 29.8 ± 5.9* | 2.5P 8/6 |
| Y2-U4-20 | 23.2 ± 14.3* | 48.0 ± 8.0* | 2.5P 8/6 and 2.5 P 10/5 |
| Y2-U5-14 | 20.7 ± 7.0* | 39.9 ± 10.5* | 2.5P 8/6 |
| Y3-U5-10 | 63.2 ± 37.1* | 48.3 ± 10.3* | 2.5P 8/6 and 10 PB 8/6 |
| Y3-U7-14 | 53.7 ± 25.8* | 41.7 ± 9.2* | 2.5P 8/6 |
| Y4-U5-9 | 53.9 ± 35.2* | 53.0 ± 10.4* | 2.5P 8/6 |
| Y4-U5-16 | 110.6 ± 38.5* | 58.9 ± 4.6* | 2.5P 8/6 |

*Significantly different with control (0 Gy) based on T-tailed test at 5%

Number of flower Callistephus chinensis at MV3 was in the range of 23 – 110 flowers. While number of flower at control treatment was 13 flowers per plant. Number of flower Callistephus chinensis cv. Shasank was 82 flowers [19]. Genotypes of Y1-U4-9, Y1-U4-11 and Y1-U5-5 had flower in the range of 78 – 86 flowers (Figure 3). Number of flower Callistephus chinensis cv. Shasank at mid hill conditions of Himachal Pradesh was 31 flowers per plant [16]. In this research, only genotype Y2-U4-20 and Y2-U5-14 had number of lower below than 31 flowers. Callistephus chinensis at MV1 that was grown at highland (915 asl) only had 2 – 5 flowers per plant [20]. There were increasing number of flower at RV1 that was planted in lowland. The reason of increasing number of flower at MV1 were effect of gamma rays irradiation and difference in climate between lowland - high land. Gamma rays irradiation inhibited plant growth at MV1. The effect of gamma rays irradiation was obvios at third generation (MV3). Gamma rays is effective mutagenic agent. Classification of mutation based on the extent of DNA sequence affected by mutation event were smale-scale mutation and large-scale mutation. Small-scale mutation is involving one or a few nucleotides such as point mutations, insertions and deletions of DNA. While, large-scale mutations affected structure of chromosome including inversions and gene duplications [21]. To make sure, whether increasing number of flower at this research because of small-scale mutation or large-scale mutation, needed intensively research of DNA sequence.
All genotype had main stalk length of flower significantly longer than control plant (Figure 4). Genotype Y4-U5-16 had the longest main stalk length of flower (58.9 cm), while genotype Y2-U3-12 had the smallest main stalk length of flower (28.9 cm). There were also increasing main stalk length of flower at MV3 that was planted in lowland compared to MV1 at highland. Main stalk length of flower at MV1 was in the range of 6.6 – 8.1 cm [20]. Flower stalk length of *Callistephus chinensis* ev. Shasank was 37.7 cm [19] and 32.6 [16]. All genotype of MV3 *Callistephus chinensis* at lowland had longer flower stalk length, except genotype Y2-U3-12. Irradiation of gamma rays increased flower stalk length of MV3 *Callistephus chinensis*. Diversity of flower stalk length only seen at genotype Y1-U4-9, Y1-U3-6 and Y2-U3-12.

**Figure 4.** Main stalk length of flower of *Callistephus chinensis* at MV3 in lowland

Flower colour of control plant was purple (2.5P 8/6). Many plant also had colour flower the same with control plant (2.5P 8/6) or violet (2.5 P 10/5). There was one replicate of genotype Y1-U4-9 had different flower colour. Colour flower of those plants (6 plants) was pink (2.5RP 6/8) (Figure 4). The change of flower colour also detected in *Torenia fournieri* and *Torenia bailloni*. Irradiation of gamma rays (90 gray) resulted one mutant with a yellow petal color, whereas the control plant had a petal with a dull yellow on the top and bottom and dark purple on the sides. Gamma rays irradiation of 50 Gy resulted in Allotetraploid *Torenia* plants. Based on cytological analysis, those mutans had chromosome number 2n=34, whereas the normal chromosome number for Torenia hybrids was 2n=17 [22]. Cytological analysis has to be done whether some plants in genotype Y1-U4-9 has different chromosome number compared to control plants.
Discoloration of *Callistephus chinensis* flower was observed during flowering. The colour of flower buds was purple. At blooming, flower colour was still the same i.e., purple. Then, flower colour gradually changed to violet and finally turned to white (Figure 5). Discoloration also occurred at some plants of genotype *Y*$_1$-*R*$_4$-9 with pink colour. Flower buds were pink, the blooming flower was still pink. Then, colour flower changed gradually to white. *Callistephus chinensis* have composite flowers which are actually flower-like inflorescences. Discoloration started from tip of ray flower, then continuously moved to edge of ray flower near the disc flower.

There were two plants that observed dwarf and early flowering. Height of those plants were only 10 cm. One plant had pink colour with 26 flowers. Another had purple with 22 flowers. Days required for flowering were 41 days (Figure 7). Cytological analysis has to be done whether those dwarf plants has different chromosome number compared to control plants.
Figure 7. Dwarf plants

4. Conclusion

*Callistephus chinensis* can adapted in lowland due to induced mutation by gamma rays. Many plant had colour flower the same with control plant (2.5P 8/6) or violet (2.5 P 10/5). There were six (6) plants that had pink (2.5RP 6/8) flower colour. There were two dwarf mutants of *Callistephus chinensis* at MVs.

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