BIODIVERSITAS
Volume 23, Number 3, March 2022
Pages: 1399-1407

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230326

Increasing the diversity of marigold (*Tagetes* sp.) by acute and chronic chemical induced mutation of EMS (Ethyl Methane Sulfonate)

DEWI YANNY LENAWATY[1,♥], DEWI SUKMA[2], MUHAMAD SYUKUR[2], DEWA NGURAH SUPRAPTA[3], WARAS NURCHOLIS[4], SYARIFAH IIS AISYAH[2,♥♥]

[1]Plant Breeding and Biotechnology Graduate Program, Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8629354, ♥email: dewiyanny@apps.ipb.ac.id
[2]Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia
[3]Universitas Udayana. Jl. Raya Kampus Unud Jimbaran, Badung 80361, Bali, Indonesia
[4]Tropical Biopharmaca Research Center, Institut Pertanian Bogor. Jl. Taman Kencana, Kampus IPB Taman Kencana, Bogor 16680, West Java, Indonesia. ♥♥email: syarifahiis@yahoo.com

Manuscript received: 1 February 2022. Revision accepted: 21 February 2022

Abstract. *Lenawaty DY, Sukma D, Syukur M, Suprapta DN, Nurcholis W, Aisyah SI. 2022. Increasing the diversity of marigold (*Tagetes *sp.) by acute and chronic chemical induced mutation of EMS (Ethyl Methane Sulfonate). Biodiversitas 23: 1399-1407.* Increasing the diversity of marigold (*Tagetes sp.*) can be carried out through chemical induction mutations with Ethyl Methane Sulfonate (EMS). This study aims to obtain the LC_{50} value, determine the sensitivity level of plants, and obtain plant diversity of *Tagetes sp.* with EMS both acutely and chronically. The genetic material used in this study was derived from *T. erecta* genotype MG04 and *T. patula* genotype MG21. The acute mutation technique was performed by soaking the seeds in EMS concentration for 4 hours under 10 different concentration levels (0%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.5%, 2.0%, and 3.0%). The chronic mutation technique was carried out by diluting the concentration of EMS LC_{50} 1/10x, 1/100x, 1/1000x and immersion time of 6 hours, 24 hours and 48 hours. The study used a Completely Randomized Block Design. Observations were made on the percentage of surviving plants, quantitative and qualitative characters. The data obtained were analyzed using Boxplot and ANOVA. The results showed that the LC_{50} value of *T. erecta* is 0.82% and LC_{50} of *T. patula* is 1.87%, which means that the sensitivity level of *T. erecta* to EMS treatment is quite higher than *T. patula*. The acute application method shows a relatively low mutation rate and limited diversity of flower types. The chronic application method produced various flower shapes and whiter flower color in *T. erecta* and *T. patula* resulting in a redder flower color than the acute application method.

Keywords: Diversity, EMS, LC_{50}, marigold, *Tagetes*

INTRODUCTION

Marigold is one of the ornamental plants that is widely planted in Indonesia. People know this plant because of its pungent aroma, known as the flower of "tahi kotok" or "telek-telekan" in Javanese and "gumitir" flower in Balinese. Marigold (*Tagetes sp*.) belongs to the Asteraceae family (Zanovello et al. 2021) and is related to chrysanthemum and sun plants (Fauziana dan Susandarini 2019), has a striking color ranging from yellow, orange to yellowish white (Bhusari et al. 2017). In the grouping there are 3 types of marigolds namely "Wild Marigold" (*Tagetes minuta*), "French Marigold" (*Tagetes patula*) and "African Marigold" (*Tagetes erecta*) (Singh et al. 2016). This study used the plant species *T. erecta* and *T. patula*.

The ornamental plant industry is growing and the demand for variations in flower colors including marigolds is relatively high. Mutation breeding can be carried out to increase diversity with good quality plant results, unique shape, and beautiful flower color. Induction mutations can be carried out using physical or chemical mutagens. X-rays, gamma rays and ultraviolet rays are included in physical mutagens (Tharek et al. 2021), while chemical mutagens commonly used in plants are EMS (Ethyl Methane Sulfonate) (Purente et al. 2020). The physical mutagens can be applied with acute irradiation and chronic irradiation technique (Aisyah 2013). The application of physical mutagens with acute techniques is performed by giving a dose at once in one irradiation shot. Whereas in chronic technique, the irradiation is given in a very low dose continuously for a longer period. Hase et al. (2020) reported that chronic gamma-ray irradiation can cause a greater genetic change than acute gamma-ray irradiation. In this study, the authors carried out acute and chronic chemical mutagen application using EMS. In the acute technique, the mutagen is given once, in a single concentration. Whereas in chronic technique, the mutagen is given in a very low concentration continuously for a longer period. Since chronic technique on EMS has never been done before, therefore the concentration range of EMS for chronic is determined from the LC_{50} of acute technique which is done previously.

One of the advantages of mutation breeding is that many species can be propagated vegetatively and sexually (Yamaguchi 2018). Chemical induction mutations using EMS have been widely used and have been shown to increase genetic diversity. EMS can also decrease plant height along with the increase of its concentrations to

sunflower plants (Cvejić et al. 2011). According to (Purnamaningsih and Hutami 2016), the EMS is often used because it is easy to be obtained in the market and its cheaper price. EMS chemical mutagens are alkaline, can produce point mutations, and change nitrogen base pairs (Talebi et al. 2012).

Some research results show that the application of EMS can produce a yellow marigold putative mutant with 0.6% EMS immersion and a concentration of 0.9% to get a chimera mutant (Pratiwi et al. 2013). Rahmah (2011) reported that immersion for 120 minutes and a concentration of 0.77%, EMS can produce mutants with large leaf stems and reddish stems in chrysanthemum plants. Until now, there is no information about Lethal Concentration 50% (LC_{50}) for *Tagetes* sp., so it is necessary to look for the LC_{50} for *Tagetes* sp. LC_{50} is also known as LD_{50}, is the dose or concentration that causes 50% of deaths in the mutagen-treated population (Mangaiyarkarasi et al. 2014). Doses in the LC_{50} range tend to produce the most mutants (Aisyah et al. 2015). The objectives of this study are to obtain the LC_{50} value, to determine the sensitivity level of plants, and obtain plant diversity of *Tagetes sp.* using EMS acute and chronic application method.

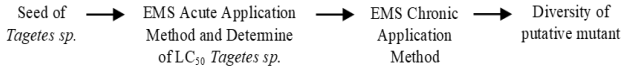
MATERIALS AND METHODS

Study area and genetic material

This research was carried out from September 2020 to July 2021 at the experimental farm of IPB Pasir Sarongge, Cianjur, West Java, Indonesia with an altitude of 1100 meters above sea level. The materials used were seeds of *T. erecta* genotype MG04 and *T. patula* genotype MG21.

Procedures

All seeds that would be used in this study, were soaked in water for 6 hours. This was done to speed up the germination process. In addition, the water immersion at this early stage is also used to select the level of seed sensitivity and viability later. Below is the workflow or flowchart of the steps on this research.



EMS acute application

The seeds were first soaked in water for 6 hours, then the seeds were soaked in EMS solution for 4 hours with a concentration level of 0% (control); 0.2%; 0.4%; 0.6%; 0.8%; 1.0%; 1.5%; 2.0%; 2.5%; 3.0%. Then the seeds were rinsed with running water five times to remove the remnants of the mutagen. The EMS-treated seeds were germinated (45 seeds for each treatment, and a total of 900 seeds for 2 genotypes). The seeds were left for 5 weeks in the seedling tray with husk, cocopeat, and manure in a ratio of 1:1:0.5 (v/v). The germination rate was observed within 2 weeks, then the LC_{50} value was calculated and used for the chronic application method. The next stage is the transfer of surviving seedlings from EMS treatment to the field and observed for their growth and morphology. Plant maintenance in the field includes watering, controlling pests and diseases, and fertilizing until the plants produce seeds.

EMS chronic application

Seeds that have been soaked in water for 6 hours, then soaked again in EMS solution. The EMS concentration administered was based on the LC_{50} obtained from the previous acute mutation trial. The concentration level consists of 4 concentration levels: 1. Without EMS (control) with 6 hours of immersion; 2. The LC_{50} concentration was diluted 1/10x with a soaking time of 6 hours; 3. The concentration of LC_{50} is diluted 1/100x with an immersion time of 24 hours; 4. The concentration of LC_{50} is diluted 1/1000x with an immersion time of 48 hours. After EMS chronic treatment, the seeds were rinsed with water five times each for 2 minutes to remove the remnants of the mutagen. Then the seeds were planted in seedling trays with the mixed medium of husk, cocopeat, and manure in a ratio of 1: 1: 0.5 (v/v). The seeds germination was observed within 2 weeks, then after 5 weeks, the surviving seedlings were transferred to the field.

Data analysis

Determination of the LC_{50} value is done by calculating the percentage of surviving plants using CurveExpert 1.4 Software. Quantitative data analysis was performed using ANOVA (Analysis of Variance) followed by DMRT (Duncan Multiple Range Test) at 5% level and boxplot analysis. Quantitative characters include plant height, canopy width, stem diameter, number of branches, flower diameter, flower length, and number of flowers. Qualitative characters have leaf type, crown shape, flower type and flower color. The qualitative characters are presented based on UPOV (The International Union for the Protection of New Varieties of Plants) *Tagetes* L. (UPOV 2007) and the Mini RHCC (*Royal Horticulture Color Chart)* is used as a reference for color coding.

RESULTS AND DISCUSSION

Seed sensitivity level

The percentage of survival plants showed that each level of EMS concentration gave a different effect on two species (Table 1). EMS concentration of 1.0% in the MG04 genotype gave a lower percentage of live plants than the 0.0% EMS concentration (control). These results indicated that the higher level of EMS concentration damaged the seed and caused the death of *T. erecta* seeds at concentration levels of 1.5%, 2.0%, 2.5%, and 3%. *T. patula* seeds could survive at any level of EMS concentration. The results of the Curve-fit analysis showed that the LC_{50} value of *T. erecta* was 0.82% (Figure 1A) and the LC_{50} value of *T. patula* was 1.87% (Figure 1B). *T. erecta* has a lower LC_{50} value range than *T. patula*. Curve-fit analysis shows that the LC_{50} value of *T. erecta* was lower than of *T. patula.* These results indicate that the

sensitivity level of *T. erecta* to EMS is higher than that of *T. patula*. The mathematical equations obtained are quadratic fit models for *T. erecta* and *T. patula* (Table 2).

EMS acute application results

The results of the Anova test on *T. erecta* showed that the EMS treatment had a significant effect on plant height, the width of canopy, number of branches, and number of flowers. Plant height at 0% concentration was higher than 0.6%, 0.8% and 1.0% (Table 3). The average plant height ranged from 26.5 cm (1.0%) to 31.28 cm (0.0%). It indicates that increasing the EMS concentration decreased plant height. In *T. patula*, the results of the Anova test showed that the EMS concentration had a significant effect on plant height, stem diameter, flower diameter, and flower number. Plant height at 0% concentration was higher than other concentration levels (Table 4). It indicated that plant height growth decreased with increasing EMS concentration. The average plant height ranged from 102.4 cm (0.0%) down to 91.0 cm (3.0%).

The effect of EMS concentration on the qualitative character of *T. erecta* plants is presented in Table 5. The observations obtained differences between individuals including parameters of flower type, flower color, and RHS code. The concentration of EMS can cause changes in the morphological characters of *T. erecta* such as canopy type and dwarf plants M1-0.4%-39, M1-0.2%-10, M1-0.8%-05 (Figure 3).

The qualitative characters of *T. erecta*, such as color and leaf type did not change, namely green color and pinnate type. However, the shape of the resulting canopy changed from semi-upright on control plants to spreading at EMS concentration levels of 0.20% and 0.60%. The shape of the flower is presented in Figure 4, at the concentration level of 0.4% and 0.8%, it produces tubulate and ligulate flower types, while at the concentration level of 0%, 0.2%, 0.6%, and 1%, it produces all ligulate flower types (Table 5). The effect of giving EMS concentration level 0.8% can produce whiter flower color than other concentration levels with RHS code N999D in M1-0.8%-19 plants (Figure 4E).

The effect of EMS concentration on the qualitative characters of *T. patula* plants is presented in Table 6. The qualitative characters of *T. patula* plants showed a difference between individual plants, including flower color parameters and RHS codes. Qualitative characters such as leaf type, canopy type, and flower type did not change. Plants M1-2.0%-37 (Figure 5H) produced a redder flower color than other concentration levels, namely the RHS code N45A.

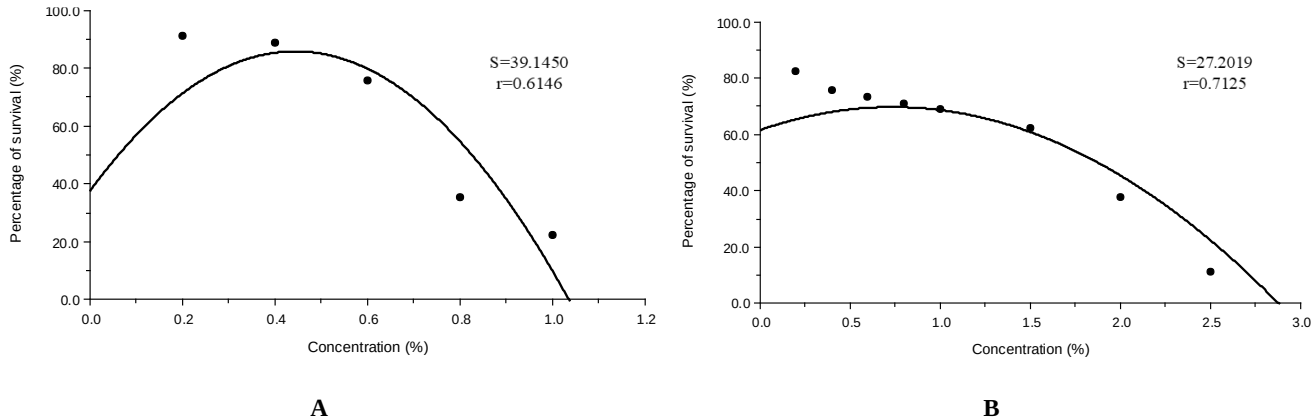
The results of the boxplot analysis of *T. erecta* (Figure 2A) showed that plants from the 0.8% EMS treatment resulted in a larger flower diameter than other plants by 7.19 cm on M1-0.8%-05 plants. The boxplot analysis on *T. patula* (Figure 2B) showed that the 2.0% of EMS treatment produced the smallest stem diameter compared to other plants, which was 1.01 cm in M1-2.0%-30 plants.

Table 1. Percentage of seed survival of *Tagetes* sp. after EMS treatment

EMS concentration (%)	Percentage of seed survival (%)	
	T. erecta	***T. patula***
0.0	100.00	100.00
0.2	91.11	82.22
0.4	88.89	75.56
0.6	75.56	73.33
0.8	35.56	71.11
1.0	22.22	68.89
1.5	0.00	62.22
2.0	0.00	37.78
2.5	0.00	11.11
3.0	0.00	2.22

Table 2. LC_{50} value of *Tagetes* sp. after EMS treatment

***Tagetes* sp.**	**Model**	**Equality**	**LC_{50} (%)**
T. erecta	Quadratic Fit $y = a + bx + cx^2$	$y = 3.75 + 2.17x – 2.44x^2$	0.82
T. patula	Quadratic Fit $y = a + bx + cx^2$	$y = 6.15 + 2.22x - 1.51x^2$	1.87



A **B**

Figure 1. Percentage of survival and EMS concentration. A. *T. erecta*, B. *T. patula*

Table 3. Effect of EMS concentration on the quantitative character of *T. erecta* under acute EMS application method

Concentration EMS %	Plant height (cm)	Canopy widht (cm)	Stem diameter (cm)	Number of branches	Flower diameter (cm)	Flower lenght (cm)	Number of flowers
0.00	31.28a	36.37a	1.10	9.61a	5.39	6.81	48.91b
0.20	28.74ab	36.19a	1.03	9.11ab	5.36	6.77	44.53b
0.40	26.28b	32.01b	0.98	8.21b	5.28	6.51	45.44b
0.60	27.46b	33.35b	1.08	9.09ab	5.37	6.47	57.35ab
0.80	25.33b	32.07b	1.11	8.00b	5.82	6.70	51.87ab
1.00	26.50b	38.50a	1.06	8.83ab	5.50	6.50	62.67a

Note: Number followed by the same letter in each parameter show are not significantly different to DMRT 5% level

Table 4. Effect of EMS concentration on the quantitative character of *T. patula* under acute EMS application method

Concentration EMS %	Plant height (cm)	Canopy widht (cm)	Stem diameter (cm)	Number of branches	Flower diameter (cm)	Flower lenght (cm)	Number of flowers
0.00	102.4a	78.00	2.59b	5.91	3.65abc	9.78	538.70a
0.20	101.0ab	78.11	2.76ab	5.84	3.92a	11.05	524.4ab
0.40	100.7ab	74.47	2.78ab	6.20	3.63abc	12.2	478.90abc
0.60	101.6ab	73.2	2.57b	5.55	3.71ab	11.35	430.70abc
0.80	99.1ab	71.06	2.89ab	6.50	3.59abc	10.94	437.80abc
1.00	100.6ab	72.67	2.86ab	5.61	3.54abc	10.44	429.40abc
1.50	98.2ab	74.47	2.59b	5.53	3.68abc	10.87	441.00abc
2.00	96.0ab	72.23	2.35b	5.85	3.57abc	13.25	325.10abc
2.50	97.0ab	73.00	3.20a	6.00	3.27cbc	10.00	293.00bc
3.00	91.0b	66.00	2.84ab	5.60	3.19c	11.60	276.80c

Note: Number followed by the same letter in each parameter show are not significantly different to DMRT 5% level

Table 5. Effect of EMS concentration on the qualitative character of *T. erecta* under acute EMS application method

Concentration (%)	Genotype	Leaf type	Canopy type	Flower type	Flower color	RHS code
0.00	M1.37	Pinnate	Semi upright	All ligulate	Light Yellow	RHS 4D
0.20	M1. 10	Pinnate	Spreading	All ligulate	White	RHS 155B
0.40	M1.24	Pinnate	Semi upright	Tubulate and ligulate	White	RHS 155C
0.60	M1.20	Pinnate	Spreading	All ligulate	White	RHS155D
0.80	M1.19	Pinnate	Semi upright	Tubulate and ligulate	White	RHS N999D
1.00	M1.13	Pinnate	Semi upright	All ligulate	White	RHS 155C

Note: Qualitative character based on UPOV 2007 *Tagetes* L. and Mini RHCC

Table 6. Effect of EMS concentration on the qualitative character of *T. patula* under acute EMS application method

Concentration (%)	Genotype	Leaf type	Canopy type	Flower type	Flower color	RHS code
0.00	M1.04	Pinnate	Upright	ligulate and tubulate	Strong Reddish Orange	RHS 42B
0.20	M1. 10	Pinnate	Upright	ligulate and tubulate	Vivid Reddish Orange	RHS 33B
0.40	M1.45	Pinnate	Upright	ligulate and tubulate	strong orange	RHS N25A
0.60	M1.32	Pinnate	Upright	ligulate and tubulate	Moderate Red	RHS N34A
0.80	M1.32	Pinnate	Upright	ligulate and tubulate	strong orange	RHS 24A
1.00	M1.31	Pinnate	Upright	ligulate and tubulate	Strong Reddish Orange	RHS 42B
1.50	M1.06	Pinnate	Upright	ligulate and tubulate	Strong Reddish Orange	RHS 42C
2.00	M1.37	Pinnate	Upright	ligulate and tubulate	Moderate Red	RHS N45A
2.50	M1.45	Pinnate	Upright	ligulate and tubulate	Strong Reddish Orange	RHS N34B
3.00	M1.16	Pinnate	Upright	ligulate and tubulate	Moderate Red	RHS N34A

Note: Qualitative character based on UPOV 2007 *Tagetes* L. and Mini RHCC

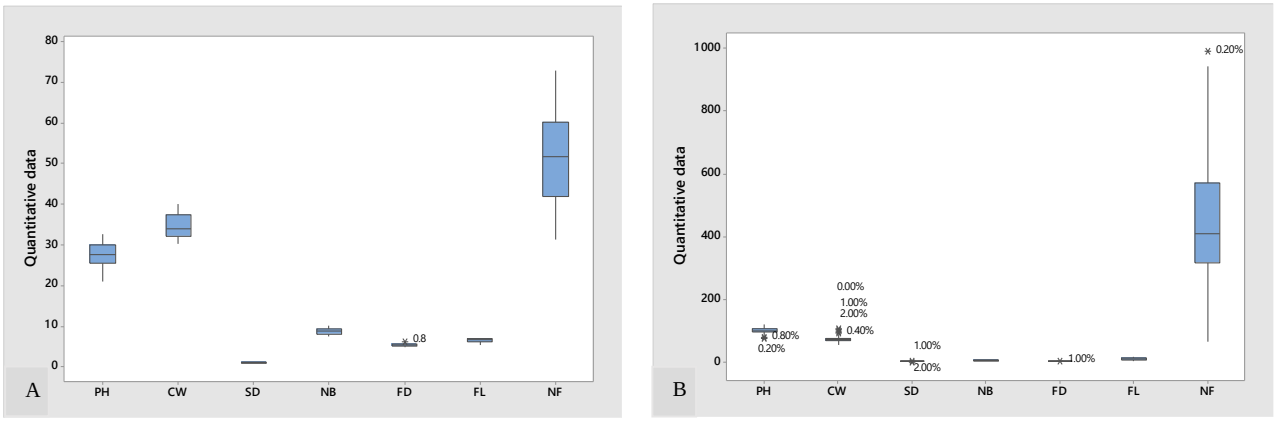


Figure 2. Boxplot analysis of quantitative characters of acute application method. A. *T. erecta.* B. *T. patula*. PH: plant height, CW: canopy width, SD: stem diameter, NB: number of branches, FD: flower diameter, FL: flower length, NF: number of flowers

Figure 3. Performance of qualitative morphology based on the shape of acute application method. A. M1-0.4%-24; B. M1-0.4%-39; C. M1-0.2%-10; D. M1-0.6%-20; E. M1-0.8%-05 at 5 weeks after field planting (WAP)

Figure 4. Performance of qualitative morphology *T. erecta* acute application method. A. Control; B. M1-0.2%-10; C. M1-0.4%-24; D. M1-0.6%-20; E. M1-0.8%-19, F. M1-1.0%-13 at 5 weeks after field planting (WAP)

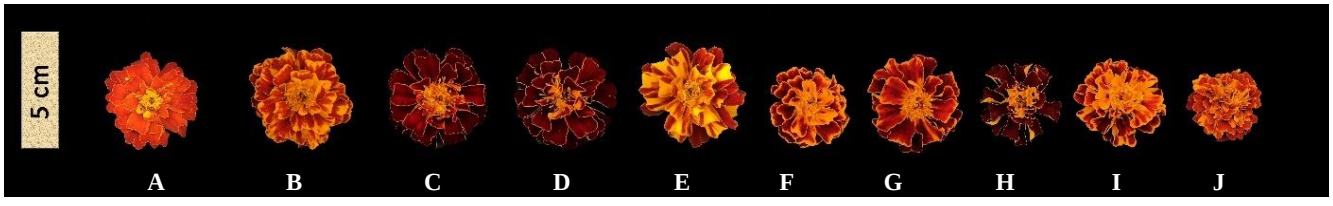


Figure 5. Performance of the qualitative morphology of *T. patula* acute application method. A. Control; B. M1-0.2%-30; C. M1-0.4%-45; D. M1-0.6%-32; E. M1-0.8%-08; F. M1-1.0%-31; G. M1-1.5%-06; H. M1-2.0%-37; I. M1-2.5%-45; J. M1-3.0%-34 at 25 weeks after field planting (WAP)

EMS chronic application results

The result from ANOVA analysis of EMS chronic treatment on *T. erecta* genotype MG04 showed a significantly different response of the plants on the characters of plant height, flower length, and the number of flowers (Table 7). The plant height at the 1/1000 treatment level was significantly higher than the control and the 1/10 EMS concentration level treatment. The flower length at the 1/100 treatment level was lower than that in the other treatment levels. The number of flowers at the 1/100 concentration level was higher than the other different treatment levels. The M1-1/100-04 plant produced a larger flower diameter (6.87 cm) and a higher number of flowers (52 flowers). ANOVA analysis of EMS chronic treatment on *T. patula* genotype MG21 showed a significant effect on plant height, the number of branches, and flower diameter. The lowest plant height was found in the 1/1000 EMS concentration level treatment soaked for 48 hours, while the control, 1/10, and 1/100 EMS concentration level treatment did not give significantly different results. In the number of branches, 1/10 EMS concentration level treatment had fewer branches than 1/100 and 1/1000, however, it was not substantially different from the control. The flower diameter characters of control plants were significantly larger than those of 1/100 and 1/1000 treatments (Table 8).

The plant of M1-1/10-28 and M1-1/100-26 showed a whiter flower color than the plants in the other treatments, with the RHS code NN155B (Figure 7). While the qualitative characters such as color and leaf type did not change in all treatments. All leaf types are pinnate types in the *T. erecta* and *T. patula*. However, the shape of the canopy was changed from semi-upright in control plants into spreading at the EMS concentration level of 1/100 and 1/1000. The shape of the flower is presented in Figure 7. At the EMS concentration level of 1/10 and 1/100, flowers shape was a tubulate and ligulate flower, while at the control and 1/1000 EMS concentration level were ligulate flower (Table 9).

The effect of EMS concentration on the qualitative characters of *T. patula* plants is presented in Table 10. The qualitative character of *T. patula* plant was different between individual plants including flower color parameters and RHS codes. At a concentration of M1-1/1000-25, the flower color is redder than that in other concentration levels, namely the RHS code N34A (Figure 8D). In the plant of M1-1/10-25 (Figure 8B), the orange tubulate is more dominant than the red ligulate. Qualitative characters such as leaf type, crown shape, and flower type did not change.

The results of the boxplot analysis of *T. erecta* test (Figure 6A) showed that the 1/100 concentration level treatment produced the smallest number of branches are 5 branches on M1-1/100-08 plants and the largest flower diameter 6.45 cm on M1-1/1000-15 plants. *T. patula* (Figure 6B) showed that the 1/1000 concentration level treatment resulted in the lowest plant height of 98 cm on M1-1/1000-25 plants and the smallest flower diameter parameter of 2.4 cm on M1-1/100-09 plants.

Table 7. Effect of EMS concentration on the quantitative character of *T. erecta* under the chronic EMS application method

Concentration EMS %	Plant height (cm)	Canopy widht (cm)	Stem diameter (cm)	Number of branches	Flower diameter (cm)	Flower lenght (cm)	Number of flowers
0.00	28.95c	35.24	0.93	7.09	5.41	7.97a	43.89b
1/10	30.86bc	36.82	0.94	7.30	5.38	8.00a	46.41b
1/100	32.29ab	37.96	1.02	6.89	5.73	6.50b	52.49a
1/1000	33.29a	38.46	1.05	7.15	5.45	7.34a	45.21b

Note: Number followed by the same letter in each parameter show are not significantly different to DMRT 5% level

Table 8. Effect of EMS concentration on the quantitative character of *T. patula* under the chronic EMS application method

Concentration EMS %	Plant height (cm)	Canopy widht (cm)	Stem diameter (cm)	Number of branches	Flower diameter (cm)	Flower lenght (cm)	Number of flowers
0.00	123.25a	109.71	2.28	8.93ab	4.03a	9.98	711.50
1/10	124.42a	112.42	2.19	8.08b	3.86ab	10.08	700.46
1/100	124.22a	113.52	2.33	9.43a	3.74b	10.14	660.90
1/1000	109.56b	113.88	2.36	9.44a	3.73b	9.31	641.00

Note: Number followed by the same letter in each parameter show are not significantly different to DMRT 5% level

Table 9. Effect of EMS concentration on the qualitative character of *T. erecta* under the chronic EMS application method

Concentration (%)	Genotype	Leaf type	Canopy type	Flower type	Flower color	RHS code
0.00	M1.14	Pinnate	Semi upright	All ligulate	Yellowish White	RHS NN155A
1/10	M1. 28	Pinnate	Semi upright	Tubuligulate and ligulate	White	RHS NN155B
1/100	M1.26	Pinnate	Spreading	Tubuligulate and ligulate	White	RHS NN155B
1/1000	M1.03	Pinnate	Spreading	All ligulate	Yellowish White	RHS NN155A

Note: Qualitative character based on UPOV 2007 *Tagetes* L. and Mini RHCC

Table 10. Effect of EMS concentration on the qualitative character of *T. patula* under the chronic EMS application method

Concentration (%)	Genotype	Leaf type	Canopy type	Flower type	Flower color	RHS code
0.00	M1.18	Pinnate	Upright	ligulate and tubulate	Strong Reddish Orange	RHS 42B
1/10	M1. 25	Pinnate	Upright	ligulate and tubulate	Moderate Red	RHS N45A
1/100	M1.08	Pinnate	Upright	ligulate and tubulate	Vivid Reddish Orange	RHS 33A
1/1000	M1.25	Pinnate	Upright	ligulate and tubulate	Moderate Red	RHS N34A

Note: Qualitative character based on UPOV 2007 *Tagetes L.* and Mini RHCC

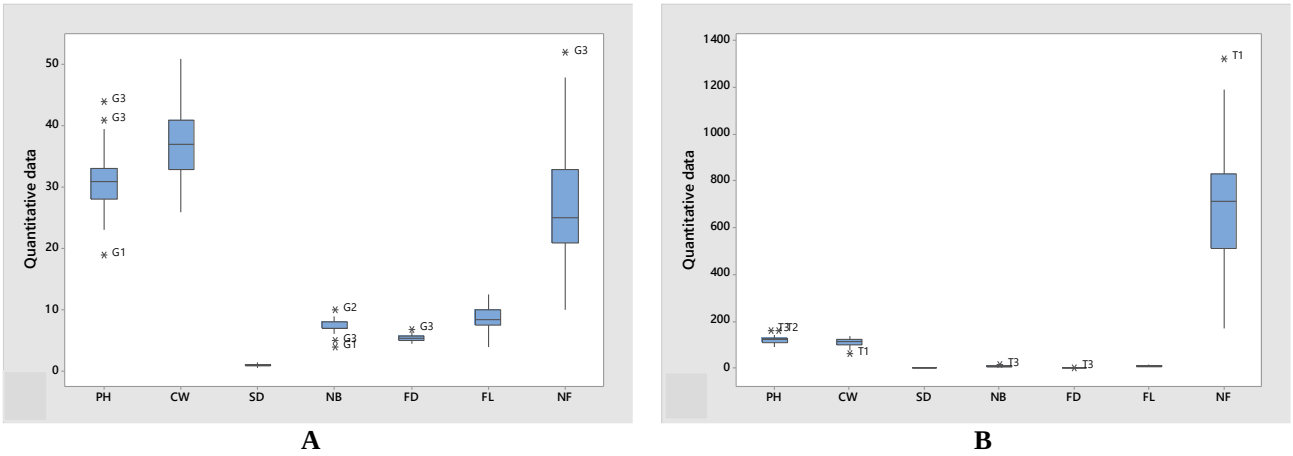


Figure 6. Boxplot analysis of quantitative characters of chronic application method. A. *T. erecta*, B. *T. patula*. PH: plant height, CW: canopy width, SD: stem diameter, NB: number of branches, FD: flower diameter, FL: flower length, NF: number of flowers

Figure 7. Performance of qualitative morphology *T. erecta* under the chronic EMS application method, A. Control; B. M1-1/10-28; C. M1-1/100-26; D. M1-1/1000-3 at 5 weeks after field planting (WAP)

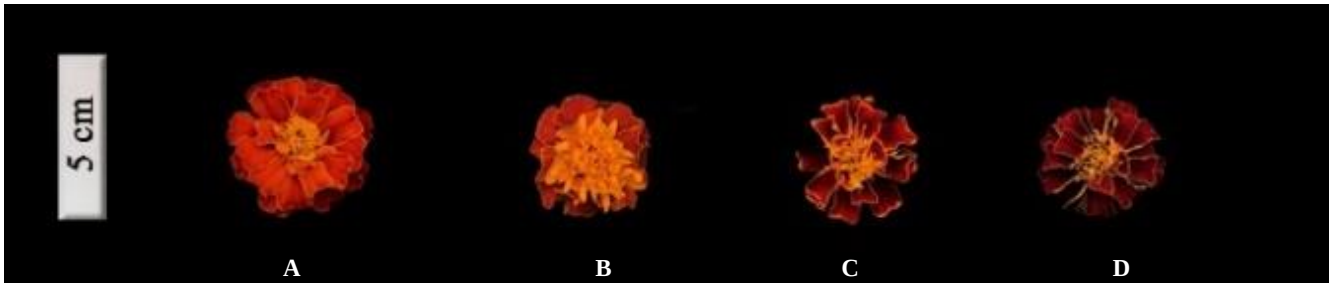


Figure 8. Performance of the qualitative morphology of *T. Patula* under the chronic EMS application method, A: Control; B. M1-1/10-25; C. M1-1/100-08; D. M1-1/1000-25 at 21 weeks after field planting (WAP)

Discussion

It is essential to determine the LC_{50} value to find the level of EMS concentration that causes 50% of the seeds to survive (Keadtidumrongkul et al. 2018). LC_{50} can be calculated based on the percentage of live plants for 2 weeks. The data was used to determine the sensitivity level of *Tagetes sp*. Kangarasu et al. (2014) stated that we must know the LC_{50} value in advance to determine the sensitivity level. Table 1 shows damaged seeds due to the higher concentration of EMS. This damage caused the death of *T. erecta* seeds at concentration levels of 1.5%, 2.0%, 2.5%, and 3%. Meanwhile, *T. patula* still germinates and survives at all levels of EMS concentration. Based on the results of this study, we can see that *T. erecta* is more sensitive to EMS than *T. patula.*

The percentage of live plants in both genotypes decreased with increasing levels of EMS concentration. This is in line with research (Eswaramoorthy et al. 2021) increasing the dose of EMS concentration reduces the percentage of live plants cowpea. Roychowdhury dan Tah (2011) also reported that the rate of germination and survival decreased as the concentration of EMS was increased in *Dianthus caryophyllus.*

Curve-fit analysis shows that the LC_{50} value of *T. erecta* was lower than of *T. patula*. These results indicate that the sensitivity level of *T. erecta* to EMS is higher than that of *T. patula*. This level of sensitivity can be influenced by the concentration of EMS and the type of genotype used. A relative of the marigold plant, sunflower seeds, produces an LC_{50} value of 0.68% (Cvejić et al. 2011). The result of Rahmah (2011) showed that the EMS concentration of 0.77% and the immersion time of 120 minutes resulted in a putative mutant with reddish stems and large leaf stems in chrysanthemum plants.

In this study, the induced diversity of *Tagetes sp*. using EMS acute application method showed that increasing EMS concentration can impact plant growth like plant height decrease. This result is supported by the research of (Rime et al. 2019) showed that the plant height of mango decreased along with the increase of the EMS concentration. Similar results were reported by (Cvejić et al. 2011) in the sunflower that EMS decreased the plant height by increasing its concentration. This phenomenon can be associated with abnormal cell division and may impair cell division and gene expression (Tarigan et al. 2021). The concentration of EMS caused the changes in the morphological characters of *T. erecta*, such as dwarf plants in M1-0.4%-39, M1-0.2%-10, and M1-0.8%-05 (Figure 3). Variations in the canopy type were found in M1-0.4%-24, M1-0.4%-39, M1-0.2%-10, M1-0.6%-20, and M1-0.8%-05. The disruption of protein synthesis can inhibit plant development. EMS chemical mutagens are alkaline, which can change nitrogen base pairs (Talebi et al. 2012).

The results of diversity of *T. erecta* are expected to meet consumer demand for ornamental plants that want unique and beautiful characters (Yamaguchi 2018), including putative mutants with different crown types from the control in M1-0.4%-24; M1-0.2%-39; M1-0.6%-10; M1-0.6%-20; and M1-0.8%-19 (Figure 4). The diversity produced from chemical mutations of *T. erecta* was found around the LC_{50} concentration of 0.8% resulting in canopy type, flower type, and the flower color is whiter than the control plants. The diversity of *T. patula* was also produced around the LC_{50} concentration of 2.0% resulting in a darker red flower color than the control plants. This phenomenon is in line with Aisyah et al. (2015), many mutants were produced at a concentration of around LC_{50}, namely at concentration levels of 0.82% and 1.87%. Research results from Pratiwi et al. (2013) showed that 0.6% EMS concentration could produce a yellow marigold flower putative mutant, and 0.9% concentration produced chimera.

In the application of acute methods, the chemical mutagen was given once, at a high concentration level therefore, the ability of plants to survive, as well as the mutant frequency created, is getting lower. For this reason, the chronic application was carried out to provide a low concentration level by diluting the dose LC_{50} and immersing seeds for a longer period. The application of a low concentration of EMS with a longer immersion time can cause most cells to obtain continuous exposure to EMS. Therefore, they become very sensitive during cell division and finally created more changes. The LC_{50} value of *T. erecta*, was 0.82% and the LC_{50} value of *T. patula* was 1.87%.

The EMS acute application shows a relatively low mutation rate, and the form of flower types is still limited. EMS Chronic application resulted in better putative mutants. In *T. erecta*, various flower shapes and colors were produced by chronic technique, whiter than those produced by acute mutation technique. In *T. patula* the flower color resulting from chronic is redder than those treated by acute application method. The new plant diversity resulting from this research has to be maintained by replanting to the next generation until producing stable mutants.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. I Wayan Koster, the Bali Province Governor, for the ideas and support for developing the marigold varities, and Office of Agriculture and Food Security of Bali Province for funding this research in collaboration with Faculty of Agriculture, IPB University, Bogor coordinated by Prof. Muhamad Syukur.

REFERENCES

Aisyah S. 2013. Mutasi Induksi. In: Syukur MS, Sastrosumardjo. Sitogenetika Tanaman. IPB Press, Bogor. [Indonesian]

Aisyah SI, Marthin Y, Damanik MRM. 2015. Improving performance of coleus through mutation induction by gamma ray irradiation. J Trop Crop Sci 2 (1): 26-32. DOI: 10.29244/jtcs.2.1.26-32.

Bhusari A, Deshmukh M, Bhagat S. 2017. Effect of gamma irradiation on morphological characters of marigold (*Tagetes erecta* L.). World J Biol Biotechnol 2 (3): 165-167. DOI: 10.33865/wjb.002.03.0109.

Cvejić S, Afza R, Jocić S, Prodanović S, Miklič V, Škorić D, Dragin S. 2011. Radiosensitivity of sunflower inbred lines to mutagenesis. Helia 34 (54): 99-106. DOI: 10.2298/HEL1154099C.

Eswaramoorthy V, Kandasamy T, Thiyagarajan K, Vanniarajan C, Jegadeesan S. 2021. Effectiveness and efficiency of electron beam in comparison with gamma rays and ethyl methane sulfonate mutagens

in cowpea. Appl Radiat Isot 171: 109640. DOI: 10.1016/j.apradiso.2021.109640.
Fauziana M, Susandarini R. 2019. Species diversity and potential use of Asteraceae in Tawangmangu, Karanganyar Regency, Central Java. J Trop Biodivers Biotechnol 4 (1): 18. DOI: 10.22146/jtbb.36652.
Hase Y, Satoh K, Seito H, Oono Y. 2020. Genetic consequences of acute/chronic gamma and carbon ion irradiation of *Arabidopsis thaliana*. Front Plant Sci 11: 366. DOI: 10.3389/fpls.2020.00336.
Kangarasu S, Ganeshram S, Joel AJ. 2014. Determination of lethal dose for gamma rays and ethyl methane sulphonate induced mutagenesis in cassava (*Manihot esculenta* Crantz.). Intl J Sci Res 3 (1): 3-6.
Keadtidumrongkul P, Chirarat N, Somran S. 2018. Determination of LD_{50} of ethidium bromide for induction of mutation in marigolds. Naresuan University J Sci Technol 26: 80-88. DOI: 10.14456/nujst.2018.24.
Mangaiyarkarasi R, Girija M, Gnanamurthy S. 2014. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in *Catharanthus roseus*. Intl J Curr Microbiol Appl Sci 3 (5): 881-889.
Pratiwi N made D, Pharmawati M, Astarini IA. 2013. Pengaruh Ethyl methane sulphonate (EMS) terhadap pertumbuhan dan variasi tanaman marigold (*Tagetes* sp.). Agrotrop 3 (1): 23-28. [Indonesian]
Purente N, Chen B, Liu X, Zhou Y, He M. 2020. Effect of ethyl methanesulfonate on induced morphological variation in M3 generation of *Chrysanthemum indicum* var. Aromaticum. HortScience 55 (7): 1099-1104. DOI: 10.21273/HORTSCI15068-20.
Purnamaningsih R, Hutami S. 2016. Increasing Al-tolerance of sugarcane using ethyl methane sulphonate and in vitro selection in the low pH media. Hayati J Biosci 23 (1): 1-6. DOI: 10.1016/j.hjb.2016.01.006.
Rahmah S. 2011. Induksi keragaman dua varietas krisan (*Dendranthema grandiflora* Tzvelev) dengan etil metana sulfonat (EMS) secara in vitro. Scientific Repository, IPB University, Bogor. [Indonesian]
Rime J, Dinesh MR, Sankaran M, Shivashankara KS, Rekha A, Ravishankar KV. 2019. Evaluation and characterization of EMS derived mutant populations in mango. Sci Hortic (Amsterdam) 254: 55-60. DOI: 10.1016/j.scienta.2019.04.015.
Roychowdhury R, Tah J. 2011. Chemical mutagenic action on seed germination and related agro-metrical traits in M1 *Dianthus* generation. Curr Bot 2 (8): 19-23.
Singh P, Krishna A, Kumar V, Krishna S, Singh K, Gupta M, Singh S. 2016. Chemistry and biology of industrial crop *Tagetes* species: A review. J Essent Oil Res 28 (1): 1-14. DOI: 10.1080/10412905.2015.1076740.
Talebi A, Talebi A, Shahrokhifar B. 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in malaysian rice (cv. MR219) for lethal dose determination. Am J Plant Sci 3 (12): 1661-1665. DOI: 10.4236/ajps.2012.312202.
Tarigan R, Hanafiah DS, Sinurya M. 2021. Radio-sensitivity test of gamma irradiation of local chilli pepper seeds (*Capsicum annum* L.). IOP Conf Ser Earth Environ Sci 782 (4): 042034. DOI: 10.1088/1755-1315/782/4/042034.
Tharek A, Yahya A, Salleh MM, Jamaluddin H, Yoshizaki S, Hara H, Iwamoto K, Suzuki I, Mohamad SE. 2021. Improvement and screening of astaxanthin producing mutants of newly isolated *Coelastrum* sp. using ethyl methane sulfonate induced mutagenesis technique. Biotechnol Rep (Amst) 32: e00673. DOI: 10.1016/j.btre.2021.e00673.
UPOV. 2007. Introduction to the international union for the protection of new varieties of plants. Workshop, Beijing, June 9 to 11, 2004.
Yamaguchi H. 2018. Mutation breeding of ornamental plants using ion beams. Breed Sci 68 (1): 71-78. DOI: 10.1270/jsbbs.17086.
Zanovello M, Bolda Mariano LN, Cechinel-Zanchett CC, Boeing T, Tazinaffo GC, Mota da Silva L, Silva DB, Gasparotto Junior A, de Souza P. 2021. *Tagetes erecta* L. flowers, a medicinal plant traditionally used to promote diuresis, induced diuretic and natriuretic effects in normotensive and hypertensive rats. J Ethnopharmacol 279: 114393. DOI: 10.1016/j.jep.2021.114393.