

Gamma Irradiation Induced High Yield Mutant Genotype Samosir Local Shallot in the Third Generation

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Received: 23 November 2020, Revised: 17 May 2021, Accepted: 23 May 2021

Abstract

The gamma irradiation can promote the agronomic characters of plant. This study aims to obtain putative mutant genotype of the 3rd generation Samosir local shallot (M_1V_3), which highly yields. The study used the augmented design in which 8 mutant genotypes of Samosir local shallot from the 2nd generation after gamma (γ)-ray treatment were planted along with untreated. The differences between the irradiation plants and untreated were tested by t-test: Similarity distance and dendrogram were analyzed using the hierarchical cluster. The results showed that almost all the mean values made by all quantitative agronomic character changes in the M_1V_3 populations were higher than those made in M_1V_2 . The expected high heritability value with a broad coefficient of genetic diversity was found in the character of fresh and dried bulb's weight resulted from genotype populations treated with γ rays irradiation with doses 3, 4 and 5 Gy. High heritability values were also found in the fresh weight and dry weight of bulb in genotype populations treated with doses 1 and 2 Gy with moderate genetic diversity coefficients. Selection based on the dried bulb's weight from each mutant genotype population with a selection intensity of 30 % was obtained 39 genotypes that showed the highest selection index. The highest number of genotypes were selected from the application of γ -rays with a dose of 1 to 2 Gy, each of 10 genotypes. Therefore, application at the dose of 1 - 2 Gy in M_1V_3 -generation for Samosir local shallot can still improve the yielding character through breeding to archive shallot in national.

Keywords: Heritability, Mutant M_1V_3 , Shallot, γ -rays irradiation

Introduction

Local Samosir shallot (*Allium ascalonicum* L.) is the horticulture plants cultivated by the smallholder in Samosir District, North Sumatra, Indonesia with 255 ha in 2018. The dominant was found in Simanindo and Pangururan Subdistricts with a productivity of 7.75 ton ha⁻¹ [1]. Shallot productivity in Samosir District is still relatively lower than the national productivity of 9.59 ton ha⁻¹ [2]. Therefore, the shallot development from Samosir District is needed to increase the high yield through the breeding program with an increase in the genetic variation.

Mugiono *et al.* [3] stated that the efforts to increase genetic variation could be conducted by crossing program, somaclonal variation, gene transformation and mutation. Hiremath *et al.* [4] stated that new superior varieties' efforts through plant breeding need to be supported by plants' high genetic variation. Genetic variation in the breeding program is one of the basic requirements for selecting the desired superior mutant genotype. The selection's effectiveness depends on the broadness of genetic variation for the character to be selected in the populations. The parameters, such as coefficient of variation and heritability can be used to determine the genetic variation in populations.

A previous study reported the low dose γ -ray irradiation 1 to 4 Gy increased genetic variation with high heritability values on several characters of the 2nd (M_1V_2) generation Samosir shallot [5]. Harten [6] stated that the selection for quantitative characters should not be carried out in the early generations before the 3rd generation because, in the earlier generation, major genes were more dominant in the plant phenotypes' appearance, making it difficult to detect mutations that occur because additive genes are still not fixed. The early generation after gamma-ray irradiation, such as those in M2, was not yet suitable for detecting mutagens' effect on genetic diversity and selection. Selection conducted in the early generation will reduce genetic variation compared to selection carried out in the later generation [6,7].

Selection of quantitative characters in the mutant population is expected to obtain selected individual plants with the desired characters in the optimum environment and adapt to stressed environments. Selection can be made using primary or secondary characters that are positively correlated with the main character. Secondary character selection can be done with 1 character or several characters at once. Selection using main characters and secondary characters will be efficient if the characters have high heritability values. Selection for multi-character could consist of 2 types: Selection based on the lowest limit value of the phenotypic character appearance (independent culling level) and selection index where the desired character is given a value weight according to the desired character level [8].

Basically, the selection in plants that reproduce asexually does not allow the emergence of increased genetic diversity between clones in the next generation. The progress of clonal selection is influenced solely by the separation of the best genotypes. In basically, each selected clone has the opportunity to be used directly as a commercial clone; this is based on the understanding that a clone's genetic constitution will not change along with vegetative propagation regeneration. The research aimed to obtain the selected genotype mutant putative on the 3rd generation of Samosir local shallot with greater agronomic characters with high yield after gamma-ray treatment.

Materials and methods

Area and design of experiment

The experiment was conducted in an experimental field on Setia Budi Street, Medan, North Sumatra Province, Indonesia. The experiment was arranged using the augmented design [9], in which 8 mutant genotypes of Samosir local shallot yielded from the previous treatment M_1V_2 generation [5], after having under gone a dormancy period of 2.5 months, were grown in rows, along with untreated, with spacing $20 \times 20 \text{ cm}^2$. Soil tillage was conducted 2 weeks before planting. The husk charcoal was applied 10 ton ha^{-1} during soil tillage by mixing it on each plot, and then all plots were given the mulch. Cow manure was applied with $50 \text{ g planting hole}^{-1}$. NPK fertilizer (15:15:15) application was at 2 and 4 weeks after planting (WAP) with 0.5 g plant^{-1} . Sprint liquid fertilizer was applied 5 mL L^{-1} at 5 to 7 WAP with the interval once a week. Pest and disease control are also conducted at the age of 5 to 7 WAP by spraying insecticide of cyromazine and the fungicide azoxystrobin + difenoconazole.

Selected in mutant M_1V_3 of Samosir local shallot

The variables such as the plant length and tiller number were observed at 6 WAP. Each plant was harvested individually, giving rise to the M_1V_3 genotype. The number of bulbs and fresh weight of bulbs were observed at harvest, and the dry weight of bulbs was determined by weighting at 2 weeks after harvest. The derivatives of M_1V_3 mutants and M_0 (un-irradiated) were dried for 2 months. The selection was conducted by selecting the best mutants that are high yield and adapting in the lowland. The selection limit for the M_1V_3 generation was calculated using the formula:

$$z = \frac{x - \mu}{\sigma^2} \quad (1)$$

Note:

$z = 1,16$ (selection intensity of 30 %).

x = Lowest value for the 30 % selection limit.

μ = Mean of the population in the M_1V_3 generation.

Data processing

Data were analyzed by calculating the average of quantitative character. The difference between the irradiation doses with the control treatment was analyzed by t-test using the Minitab v.16 software. Data analysis was also conducted by calculating the phenotypic variance, genotype variance, heritability and genetic variation coefficient. Genetic variation in the M_1V_3 generation was calculated using the formula:

$$\sigma^2 = \frac{(\sum x^2) - [(\sum x)^2/n]}{n - 1} \quad (2)$$

Note:

$\sigma^2 M_3 = \sigma^2 p$.

$\sigma^2 p = \sigma^2 g + \sigma^2 e$.

$\sigma^2 g = \sigma^2 p - \sigma^2 e = \sigma^2 M_3 - \sigma^2 M_0$.

σ^2 = variance.
 n = number of populations.
 σ^2_p = phenotypic variance.
 σ^2_g = genotype variance.
 σ^2_e = environmental variance.
 $\sigma^2_{M_3}$ = populations variance of M_1V_3 generation.
 $\sigma^2_{M_0}$ = populations variance of M_0 (un-irradiated).

Heritability value was calculated using the formula [10]:

$$h^2 = \sigma^2_g / \sigma^2_p \quad (3)$$

Heritability value criteria: $h^2 > 0.5$ (high), $0.2 < h^2 < 0.5$ (moderate) and $h^2 < 0.2$ (low). The highest CGV absolute value was determined from CGV relative value of 100 %. Genetic variation was determined based on the coefficient of genetic variation (CGV) using the formula:

$$CGV = \frac{(\sigma_g)}{\bar{x}} \times 100 \% \quad (4)$$

Note:

CGV = coefficient of genetic variation.

σ_g = square root of genotype variance.

\bar{x} = average property values.

Similarity distance and dendrogram analysis of 39 mutants M_1V_3 of Samosir local shallot was performed based on the plant length, number of tillers, number of bulbs, fresh- and dry weight of bulbs using the hierarchical cluster analysis with IBM SPSS Statistics v.20 software.

Results and discussion

Agronomic and yield characters in mutant M_1V_3 of Samosir local shallot

Low dose irradiation treatment resulted in a different population from the control population for the character of plant length, number of tillers, number of bulbs, fresh weight of bulbs and bulb dry weight of bulbs in mutant M_1V_3 of Samosir local shallot (**Table 1**). Changes in the mean value of plant length characters were found in the irradiated population of 1 and 2 Gy doses and were significantly different from the control. Likewise, changes in the tiller number's mean value were in line with changes in the mean values of fresh weight and dry weight characters in the irradiated population of doses 1, 2 and 3 Gy, which significantly differed from the control population.

The mean values of plant length, tiller number, bulb number, bulb fresh weight, and bulb dry weight increased significantly in the irradiated population of 1 and 2 Gy doses compared to the control population. The population resulting from irradiation doses of 1 and 2 Gy was the population that experienced the most changes in the mean character value than other irradiated populations. Observations of the 8 irradiated populations in the M_1V_3 generation showed that the mean value of higher agronomic characters was made by the population irradiated at a dose of 2 Gy was higher than other irradiated populations. This result indicates that in the population caused by plants after irradiated with 2 Gy, a large number of individuals had higher plant height, tiller number and bulb number.

Irradiation at the dose of 1 until 3 Gy significantly increased the character dry weight of bulbs in mutant M_1V_3 of Samosir local shallot compared to the un-irradiated population. Irradiation at the dose of 1 until 4 Gy significantly increased the fresh weight of bulbs in mutant M_1V_3 of Samosir local shallot compared to the un-irradiated population. Irradiation at the dose of 1 to 3 Gy significantly increased the number of tillers in mutant M_1V_3 of Samosir local shallot compared to the un-irradiated population.

Table 1 The agronomic and yield characters in mutant M_1V_3 of Samosir local shallot after γ -irradiated.

Agronomic and yield characters (\pm S.D.)					
Irradiation doses (Gy)	Plant length at 6 WAP (cm)	Number of tillers at 6 WAP	Number of bulbs	Fresh weight of bulbs (g)	Dry weight of bulbs (g)
0	29.63 \pm 3.63	4.94 \pm 1.58	5.70 \pm 1.96	30.94 \pm 9.49	26.90 \pm 8.10
1	32.88 \pm 4.01**	5.93 \pm 1.94**	7.11 \pm 2.31**	45.10 \pm 15.40**	37.90 \pm 13.50**
2	33.62 \pm 4.09**	6.40 \pm 1.96**	7.60 \pm 2.57**	44.20 \pm 17.00**	41.02 \pm 12.05**
3	29.95 \pm 3.83 ns	5.94 \pm 1.96**	6.42 \pm 2.40 ns	38.70 \pm 18.90*	34.40 \pm 16.60*
4	29.34 \pm 6.46 ns	5.44 \pm 1.78 ns	6.56 \pm 2.40 ns	36.60 \pm 16.40*	30.80 \pm 14.70 ns
5	29.95 \pm 4.44 ns	5.33 \pm 1.86 ns	6.19 \pm 1.98 ns	33.50 \pm 14.10 ns	29.20 \pm 12.10 ns
6	29.50 \pm 3.98 ns	4.75 \pm 1.82 ns	5.33 \pm 2.23 ns	26.80 \pm 12.50 ns	24.20 \pm 11.20 ns
8	28.61 \pm 4.51 ns	3.43 \pm 1.13*	4.43 \pm 1.62 ns	22.36 \pm 9.53 ns	19.69 \pm 8.24 ns
9	25.80 \pm 6.55 ns	3.20 \pm 1.10*	4.20 \pm 1.30 ns	17.30 \pm 11.90 ns	14.70 \pm 10.20 ns

Note: * and ** = significantly different from the un-irradiated population (0 Gy) at the level of 5 and 1 %, ns = not significant based on the t-test and S.D. = standard deviation.

Bulbs after γ -rays irradiation at dose 2 Gy showed the significantly increased and the higher in plant length, number of tillers, number of bulbs and dry weight of bulbs in mutant M_1V_3 of Samosir local shallot around 13.47, 29.55, 33.33 and 52.49 %, respectively compared to the un-irradiated population (**Table 1** and **Figure 1**). The highest of the bulbs' dry weight in mutant M_1V_3 of Samosir local shallot was found in the dose of 1 Gy, about 45.77 % higher than the un-irradiated population. It indicated that the low dose of gamma-rays 1 to 2 Gy is preferable in promoting the growth and yield in mutant M_1V_3 of Samosir local shallot than the high doses (3 until 9 Gy). It is linear with the heritability value on plant length, tiller number, and bulb number in mutant M_1V_3 of Samosir local shallot at the dose of 2 Gy γ -irradiation classified as moderate, and the character bulb fresh and bulb dry weight classified as high (**Table 2**).

Overall, the results obtained in the 3rd M_1V_3 generation were significantly different from those of the 1st M_1V_1 [11] and the 2nd M_1V_2 generation [5]. In the M_1V_1 and M_1V_2 generations, the irradiated population's mean value for all observed characters was lower than the un-irradiated plant population's mean value. According to Wi *et al.* [12], the increased slightly seedling growth on *Arabidopsis* was exposed to γ -ray at the dose of 1 or 2 Gy and was decreased at the high dose of 50 Gy compared to un-exposed γ -ray. Abdullah *et al.* [13] also stated that the increased dry weight of rhizome in *Zingiber officinale* Roscoe of Tanjung Sepat cultivar at the dose of 5 Gy γ -ray of 18.62 % compared to the un-exposed and had decreased with increasing γ -ray doses up to 13 Gy.

The character changes in mutant M_1V_3 of Samosir local shallot compared to the untreated are displayed (**Figure 1**). An increase in the plant length, the number of tillers, the number of bulbs, fresh and dry weight of bulbs in mutant M_1V_3 of Samosir local shallot at the doses of 1 to 2 Gy γ -irradiation. However, it decreased with increasing doses of γ -irradiation compared to the un-irradiated.

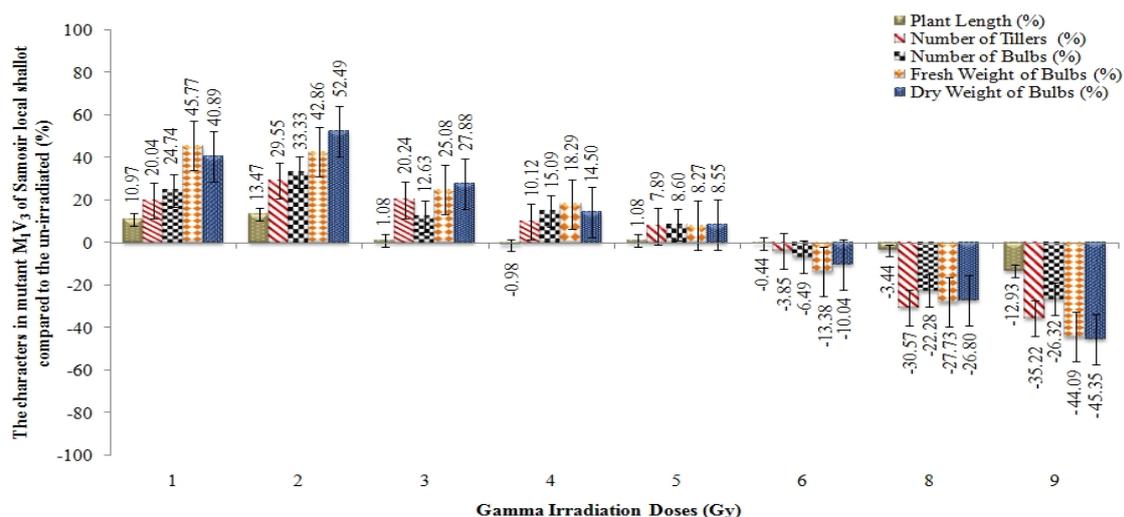


Figure 1 The characters changes in mutant M_1V_3 of Samosir local shallot were gamma-irradiated compared to the un-irradiated. Vertical bars indicate standard error.

Figure 1 showed an increase in the characters of agronomic and yield in mutant M_1V_3 of Samosir local shallot at the low dose of γ -irradiation. However, it decreased with increasing doses of γ -irradiation compared to the untreated. It was caused by the higher dose of γ -ray, resulting in changes in the physiological, biochemical, genetic and cytological will, which decrease the yield of Samosir local shallot. It is evidenced by the decrease in shallot yield (number of bulbs, fresh- and dry weight of bulbs) from doses of 2 up to 9 Gy (**Table 1**). The performance of sample selected treated plants yielded in the M_1V_3 generation could be presented in **Figure 2**.



Figure 2 The performance of sample selected treated plants yielded in M_1V_3 generation: Untreated plant (A), treated with 1 Gy (B) and treated with 2 Gy γ -rays irradiation (C).

According to Gunkel and Sparrow [14], the γ -ray can affect plants' growth and development by inducing the cytology, genetic, biochemical, physiological and morphogenetic changes in cells and tissues. Kumar and Ratnam [15] stated the decrease in sunflower life percentage in varieties USH-430 and SHSF-333 with increasing dose of γ -ray. Celik *et al.* [16] reported that the reduction in the protein content, chlorophyll content (a, b and total), carotenoid and leaf area total of soybean plant irradiated with 300 Gy γ -ray as much 44.47, 80.12, 58.64, 69.51, 81.08 and 98.86 %, respectively compared to the un-irradiated at 14 days after irradiated. Afrin *et al.* [17] also found an increase in shallot growth and yield at the dose of 0 up to 75 Gy but decreased at 100 Gy. The dose of 75 Gy γ -irradiation was produced the highest germination (79.33 %), plant height, number of leaves plant⁻¹, bulbs diameter, dry weight of bulbs, bulbs length, leaf chlorophyll content (7.45 $\mu\text{mol m}^{-2}$), protein content (11.20 %) and bulbs yield (17.47 ton ha⁻¹) compared to the un-irradiated.

Phenotypic, genotypic and heritability in mutant M₁V₃ of Samosir local shallot

Table 2 displayed the estimation of the phenotype and genotype variance, heritability and coefficient of genetic variation in the character mutant M₁V₃ of Samosir local shallot. The results showed that only the fresh- and dry weight of bulbs in M₁V₃ Samosir local shallot had high heritability values, i.e., 55 until 75 % and 55 until 76 %, respectively, at the doses of 1 to 5 Gy. The result indicated that the genetic factors dominant role in the characters fresh- and dry weight of bulbs in mutant M₁V₃ of Samosir local shallot compared to environmental factors. The selection is classified as effective.

Table 2 Phenotype variance, genotype variance, heritability and coefficient of genetic variation in the character mutant M₁V₃ of Samosir local shallot at the dose of 1 to 9 Gy γ -irradiation.

Gamma-ray doses (Gy)	Characters	σ^2_p	σ^2_g	h^2	CGV
1	Plant length	16.09	2.92	0.18 (L)	5.20 (N)
	Number of tillers	3.75	1.27	0.34 (M)	19.03 (W)
	Number of bulbs	5.34	1.49	0.28 (M)	17.18 (W)
	Fresh weight of bulbs	241.31	151.24	0.63 (H)	28.71 (M)
	Dry weight of bulbs	182.27	116.59	0.64 (H)	28.49 (M)
2	Plant length	16.74	3.57	0.21 (M)	5.62 (N)
	Number of tillers	3.84	1.36	0.35 (M)	18.20 (W)
	Number of bulbs	6.59	2.74	0.42 (M)	21.77 (W)
	Fresh weight of bulbs	238.54	148.46	0.62 (H)	26.09 (M)
	Dry weight of bulbs	174.66	108.98	0.62 (H)	25.33 (M)
3	Plant length	14.63	1.46	0.10 (L)	4.04 (N)
	Number of tillers	3.82	1.34	0.35 (M)	19.49 (W)
	Number of bulbs	5.74	1.89	0.33 (M)	21.40 (W)
	Fresh weight of bulbs	356.63	266.56	0.75 (H)	42.20 (W)
	Dry weight of bulbs	276.60	210.93	0.76 (H)	42.17 (W)
4	Plant length	41.74	28.57	0.68 (H)	18.22 (W)
	Number of tillers	3.16	0.68	0.21 (M)	15.11 (W)
	Number of bulbs	5.75	1.90	0.33 (M)	21.03 (W)
	Fresh weight of bulbs	268.08	178.01	0.66 (H)	36.45 (W)
	Dry weight of bulbs	215.89	150.21	0.70 (H)	39.78 (W)
5	Plant length	19.74	6.57	0.33 (M)	8.56 (M)
	Number of tillers	3.46	0.98	0.28 (M)	18.56 (W)
	Number of bulbs	3.92	0.07	0.02 (L)	4.35 (N)
	Fresh weight of bulbs	199.11	109.04	0.55 (H)	31.16 (W)
	Dry weight of bulbs	145.79	80.11	0.55 (H)	30.64 (W)
6	Plant length	15.81	2.63	0.17 (L)	5.50 (N)
	Number of tillers	3.30	0.81	0.25 (M)	19.00 (W)
	Number of bulbs	4.97	1.12	0.23 (M)	19.85 (W)
	Fresh weight of bulbs	156.65	66.58	0.43 (M)	30.40 (W)
	Dry weight of bulbs	124.67	59.00	0.47 (M)	31.76 (W)
8	Plant length	20.35	7.18	0.35 (M)	9.37 (M)
	Number of tillers	1.29	-1.20	0.00 (L)	0.00 (N)
	Number of bulbs	2.62	-1.23	0.00 (L)	0.00 (N)
	Fresh weight of bulbs	90.76	0.69	0.01 (L)	3.72 (N)
	Dry weight of bulbs	67.90	2.22	0.03 (L)	7.57 (N)
9	Plant length	42.97	29.80	0.69 (H)	1.16 (N)
	Number of tillers	1.20	-1.28	0.00 (L)	0.00 (N)
	Number of bulbs	1.70	-2.15	0.00 (L)	0.00 (N)
	Fresh weight of bulbs	141.05	50.98	0.36 (M)	41.32 (W)
	Dry weight of bulbs	103.52	37.85	0.37 (M)	41.96 (W)

Note: σ^2_p = phenotype variance, σ^2_g = genotype variance, h^2 = heritability (L = low, M = moderate and H = high) and CGV = coefficient of genetic variation (N = narrow, M = moderate and W = wide).

According to [18-20], the estimation of heritability value was used to determine the role of genetic factor relative to environmental factors in providing the phenotype appearance. Hakim [21] stated that the heritability value is very useful in the selection process. Selection will be effective if the population has the value of wide genetic variation and high heritability. Roy [8] stated that the variation controlled by genetic factors mostly determines the selection success. Sa'diyah [22] stated that the high heritability value followed by the high genetic variation value indicates the character appearance is more determined by genetic factors.

The high heritability value with a wide coefficient genetic variation was found in the character of the fresh- and dry weight of bulbs with a dose of 3 until 5 Gy γ -irradiated. The high heritability value was also found in the fresh- and dry weight of bulbs with a dose of 1 to 2 Gy γ -irradiated. However, the coefficient of genetic variation was classified as medium. Therefore, the selection character was used is the dry weight of bulbs (the bulbs are dry well-aerated at 2 weeks after harvest). The determined that is continued in the next stage, the selection was conducted by selecting the mutant genotypes with high yield in the wide coefficient of genetic variation and the high heritability.

Agronomic performance of mutant genotype M₁V₃ of Samosir local shallot selected

The selected plant numbers at the 30 % selection limit based on the irradiated population are presented in **Table 3**. From the selection results based on the lowest limit value of good agronomic character appearance and high yield, 39 putative mutant genotypes were obtained. **Table 3** shows that the larger number of selected genotypes was made by 1 and 2 Gy irradiated plant populations. In the plant population irradiated with a dose of 9 Gy, no genotype was selected because the bulb weight produced by the plant population was below the minimum value limit.

Table 3 Performance of agronomic characters from 39 genotypes selected in mutant M₁V₃ of Samosir local shallot at the selection limit of 30 % based on the dry weight of bulbs.

γ -ray doses (Gy)	Genotypes number	Plant length	Number of tillers	Number of bulbs	Fresh weight of bulbs (g)	Dry weight of bulbs (g)
1	M.1.4.2	32.6	7	8	62.4	54.5
	M.1.4.3	35.1	6	6	64.4	58.3
	M.1.4.4	30.2	7	8	62.0	57.2
	M.1.5.4	29.5	7	9	71.7	65.2
	M.1.6.4	35.4	8	10	64.9	59.1
	M.1.7.1	34.8	6	10	68.9	62.4
	M.1.12.2	35.3	7	9	59.1	53.8
	M.1.17.1	36.8	9	12	79.5	66.8
	M.1.17.3	40.4	11	13	81.2	64.1
	M ₁ V ₃ -1.18.6	36.7	10	11	69.6	58.8
The minimum limit selection of the dry weight of bulb was 53.56 g plant ⁻¹						
2	M.2.6.7	38.9	8	10	65.7	56.0
	M.2.9.7	38.2	9	11	83.0	73.5
	M.2.13.6	40.8	8	11	77.2	68.6
	M.2.15.4	33.4	8	8	65.8	57.5
	M.2.15.6	35.7	8	11	68.8	63.3
	M.2.16.1	37.3	8	10	66.0	57.1
	M.2.16.4	36.3	8	7	62.1	55.5
	M.2.16.5	34.5	7	12	61.1	55.2
	M.2.18.2	38.2	9	12	67.9	60.4
	M.2.19.6	35.2	10	10	62.1	58.2
The minimum limit selection of the dry weight of bulb was 54.99 g plant ⁻¹						
3	M.3.2.1	29.8	8	12	71.8	64.0
	M.3.11.4	32.2	9	10	74.2	67.1
	M.3.11.5	34.5	11	13	92.1	83.4
	M.3.12.8	33.3	7	10	64.6	58.2
	M.3.13.2	33.0	8	9	68.8	60.5
The minimum limit selection of the dry weight of bulb was 53.73 g plant ⁻¹						

γ -ray doses (Gy)	Genotypes number	Plant length	Number of tillers	Number of bulbs	Fresh weight of bulbs (g)	Dry weight of bulbs (g)
4	M.4.6.4	32.3	9	13	74.0	5.5
	M.4.6.8	34.2	6	8	54.5	48.8
	M.4.7.2	35.6	5	5	55.9	50.2
	M.4.7.5	33.4	6	10	59.7	52.7
	M.4.7.7	35.4	7	7	57.0	53.3
	M.4.7.8	30.7	7	9	59.3	58.0
The minimum limit selection of the dry weight of bulb was 47.85 g plant ⁻¹						
5	M.5.1.5	32.9	7	10	56.4	47.1
	M.5.4.5	30.5	9	10	49.9	43.3
	M.5.5.1	35.0	7	8	49.9	43.2
	M.5.8.1	29.6	6	10	56.9	49.5
	M.5.10.5	31.2	8	11	59.8	53.4
The minimum limit selection of the dry weight of bulb was 43.24 g plant ⁻¹						
6	M.6.2.5	32.1	7	9	53.7	47.9
	M.6.3.4	32.1	6	9	44.7	40.4
The minimum limit selection of the dry weight of bulb was 37.19 g plant ⁻¹						
8	M.8.1.3	34.2	4	3	33.3	30.2
The minimum limit selection of the dry weight of bulb was 29.24 g plant ⁻¹						

Note: M.1.4.2 = M (mutant), 1 (γ -ray doses), 4 (column number) and 2 (genotypes number).

The selection of bulb weight characters in the M_1V_3 population resulted in the highest selected mean value of the mutant genotype irradiated with a dose of 3 Gy with a mean value of 83.4 g, which was almost 3 times heavier than the average bulb weight of untreated plants, which was only 26.9 g. The result of high bulb weight selection was also made by the mutant genotype irradiated with a dose of 2 Gy with the selected mean value was 73.5 g. Selection based on quantitative properties is expressed in numerical form, thus it requires observation and measurement. The expression of quantitative traits is greatly influenced by environmental factors, and this is because quantitative traits are controlled by many genes (polygenic). In M_1V_3 generation, the mean value of plant length as well as the number of tiller and bulb, and bulb weight of the plant population irradiated with 1 and 2 Gy were significantly higher than the untreated plants. These results indicate a close relationship between plant length, number of tillers and bulbs with bulb weight produced. The longer the plant and the greater the number of tillers, the wider the total surface area of the leaves that results in photosynthesis, and the more photosynthetic products stored in the bulbs and the higher the weight of the resulting bulbs. The number of tillers was correlated with the number of bulbs because each good tiller produced 1 bulb, and the number of bulbs was correlated with bulb weight.

Hierarchical cluster analysis in mutant M_1V_3 of Samosir local shallot

The similarity distance between 39 selected mutants of Samosir local shallot were relatively close (Table 4). The similarity distance between 39 selected mutants of the 3rd generation (M_1V_3) of Samosir local shallot were relatively close. The nearest similarity distance was found in mutant M3.11.5 with M3.12.8 of 0.80, that the characters of the 2 genotypes are increasingly similar. The hierarchical cluster analysis was showed that only one of 39 mutants M_1V_3 of Samosir local shallot, M.8.1.3 mutant, was classified as 1 cluster with the un-irradiated population. In comparison, 38 mutants had the great agronomic and yield characters compared to the un-irradiated.

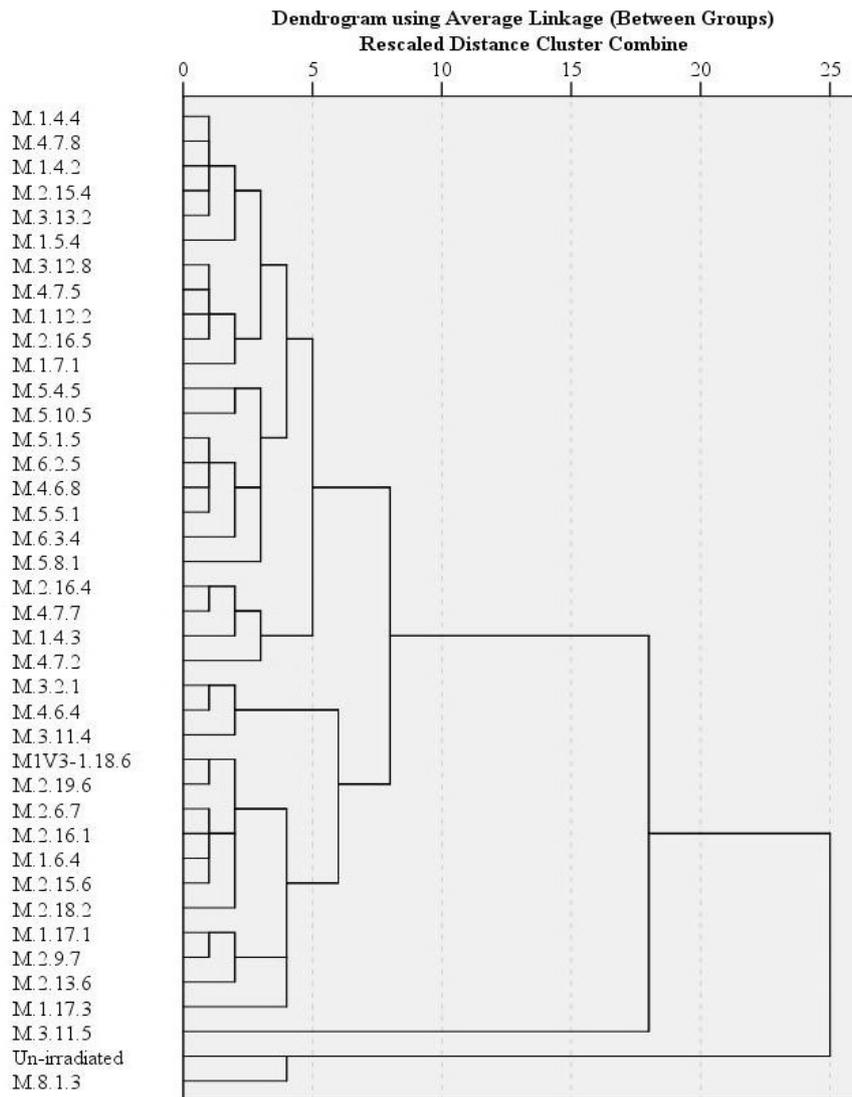


Figure 3 Dendrogram of 39 genotypes selected in mutant M_1V_3 of samosir local shallot based on the characters of plant length, number of tillers, number of bulbs, fresh- and dry weight of bulbs using the hierarchical cluster analysis.

The nearest similarity distance showed the characters of the genotypes are increasingly similar. The hierarchical cluster analysis showed that 38 of 39 mutants M_1V_3 of Samosir local shallot had great agronomic and yield characteristics compared to the un-irradiated (**Figure 3**). According to Parry *et al.* [23], the 3rd generation (M3) population is segregated, resulting in not all plants in the M₃ generation experience the character changes inherited from the 2nd generation. In addition, Sianipar *et al.* [24] stated that the γ irradiation with the dose of 6 Gy increased the number of shoots, the number of leaves and the plant height of *Typhonium flagelliforme* Lodd in the 3rd generation (MV3) compared to the un-irradiated (control). Based on the 17 clones planted from the 1st generation (MV1), 1 clone showed 100 %, 3 clones 78 %, 9 clones 56 % and 4 clones 33 % genetic similarity compared to the control plants in the 3rd (MV3) generation.

Conclusions

Gamma irradiation significantly increased the plant length, number of tillers, number of bulbs, fresh- and dry weight of bulbs in mutant M_1V_3 of Samosir local shallot at the dose 1 and 2 Gy. Gamma irradiation with 2 Gy could increase the plant length, number of tillers, number of bulbs and dry weight of bulbs in mutant M_1V_3 of Samosir local shallot about 13.47, 29.55, 33.33 and 52.49 %, respectively compared to the un-irradiated. The fresh- and dry weight of bulbs in mutant M_1V_3 of Samosir local shallot had high heritability values of 55 to 75 % and 55 to 76 %, respectively, at dose 1 - 5 Gy. A selection intensity of 30 % based on each mutant genotype population's by tuber dry weight, 39 genotypes were obtained, which showed the highest selection index. The 10 highest genotypes were selected at a dose of 1 - 2 Gy of γ -rays. Therefore, the application of γ -rays at a dose of 1 - 2 Gy in the 3rd generation to Samosir local shallots can be used as an effort to find superior mutants through increasing genetic variation for production that is sufficient for national shallot needs.

Acknowledgements

This research was supported by the Ministry of Research, Technology, and Higher Education (Kemenristek DIKTI), Indonesia, for supporting financial assistance through the dissertation grant program.

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