NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro; doi.org/10.59665/rar4003
ROMANIAN AGRICULTURAL RESEARCH, NO. 40, 2023
Print ISSN 1222-4227; Online ISSN 2067-5720

GENETIC VARIABILITY, HERITABILITY AND SELECTION OF M2 SORGHUM SUPER 2 MUTANT LINES DERIVED FROM IRRADIATION USING GAMMA RAYS

Endang Gati Lestari[1], Iswari Saraswati Dewi[1], Rossa Yunita[2*]

[1]Research Center for Food Crops, National Research and Innovation Agency
Cibinong Science Center, Jl. Raya Jakarta-Bogor, KM46, Cibinong-Bogor 16911 West Java-Indonesia
[2]Research Center for Horticulture and Estate Crops, National Research and Innovation Agency
Cibinong Science Center, Jl. Raya Jakarta-Bogor, KM46, Cibinong-Bogor 16911, West Java-Indonesia
[*]Corresponding author. E-mail: rossa_yunita@yahoo.com

ABSTRACT

Sweet sorghum (*Sorghum bicolor* L. Moench) is a potential plant to be developed as food, feed, and industrial raw materials. In Indonesia, sweet sorghum breeding is needed because only five varieties of sweet sorghum have been released. Genetic variability is essential in breeding. Through mutation induction, breeders can increase genetic variability so that new characters superior to their parents can be obtained. This study aimed to analyze the effect of various doses of gamma-ray irradiation on genetic variability in the agronomic character of the M2 population derived from the sweet sorghum Super 2 mutant and to select potential populations for further selection. The research was carried out at Experimental Garden, ICABIOGRAD, in February-June 2021. The material used was selected M2 mutant seeds from mutant populations resulting from gamma-ray irradiation of sorghum's shoots of Super 2 variety with doses of 40, 50, 60, and 70 Gy. Variables observed were plant height, stem diameter, panicle length, panicle diameter, sugar content, and fresh and dry panicle weights. The results showed that all radiation doses could increase genetic variability in M2 plants compared to their parents. Significant differences in characters between the original parents and the M2 population with a dose of 40 Gy were seen in the top diameter of the stem, panicle length, and sugar content; at a dose of 50 Gy in panicle diameter, sugar content, and fresh panicle weight; at a dose of 60 Gy on plant height, top stem diameter, sugar content, fresh panicle weight; while at a dose of 70 Gy on bottom stem diameter and middle stem diameter. The potential population for further selection based on fresh weight is the irradiated mutant population with a dose of 70 Gy. In pre-analysis using Cluster Gram and 49 mutant genotypes of the 70Gy M2 population, a group of mutants similar to their parent (Super 2 sorghum variety) and the other group that was completely different from their parent was obtained.

Keywords: agronomic character, sweet sorghum mutant, mutation, physical mutagen.

INTRODUCTION

Sweet sorghum is sorghum (*Sorghum bicolor* L.), a food plant with a short life cycle. Its advantages are broad agro-ecological adaptability, tolerance to drought, salinity and drought, fast growth, high sugar content, and biomass, and can be ratified (Almodares et al., 2011). Therefore, sweet sorghum adapts to climate change.

Sweet sorghum is a multifunctional and zero-waste product because almost all plant parts can be used as food, feed, and industry (Reddy et al., 2005; Rao et al., 2013). Sorghum is a functional food because it does not contain gluten, has a low glycemic index, and has high antioxidants and fiber (Damardjati et al., 2000; Suarni, 2012; Suarni, 2016). Seeds, leaves, and stems can be processed into animal feed as fresh or silage (Londra and Sutami, 2018; Harmini, 2021). The difference between sweet sorghum and ordinary sorghum is that the stem of sweet sorghum contains more sap. The sap in sweet sorghum contains sugar consisting of sucrose, glucose, fructose, and maltose, so sweet sorghum can be used as a raw material for the industry of fresh drinks, syrup, molasses, monosodium glutamate, and ethanol (Ratnavathi et al., 2011).

The genetic diversity of sorghum in Indonesia is limited because sorghum is not

Received 6 September 2022; accepted 12 January 2023. First Online: January, 2023. DII 2067-5720 RAR 2023-34

native to Indonesia. Recently, sweet sorghum widely planted in Indonesia, such as in Java, South Sulawesi, NTB, and NTT, which have dry and rainfed land. However, the varieties used are still limited because there are only five varieties of sweet sorghum, namely Super 1, Super 2, Bioguma 1, Bioguma 2, and Bioguma 3. Therefore, the development of new varieties of sweet sorghum is needed to support the food, feed, and bioenergy industry.

The main activity in breeding is identifying important characters (Oladosu et al., 2016). Breeding activities can be carried out if there is genetic variability in the population so that the desired important character can be selected (Laskar et al., 2015). Genetic mutation is a technique to increase genetic variability needed to obtain superior traits, such as high production and resistance to biotic and abiotic stresses (Roychowdhury and Tah, 2011; Nouri and Tavasolli, 2012; Arisha et al., 2015; Oladosu et al., 2016; Raina et al., 2017; Raina et al., 2018; Verma et al., 2018; Lestari et al., 2019).

Mutation techniques have been widely applied to increase genotype variability in characters of plants with high economic value, such as ornamental plants, vegetables, and fruit (Arisha et al., 2015; Laskar et al., 2015; Jankowicz-Cieslak et al., 2016; Lestari, 2021). Changes due to mutations can occur at the gene or chromosome level (Lestari, 2021). The advantages of using mutation induction are (i) the ability to obtain a new character in a plant variety without causing changes in other characters of the initial parent plant, and (ii) obtaining new gene alleles that are not in the existing germplasm (Khah et al., 2015).

Physical mutagens using gamma rays are more widely used than chemical mutagens to induce mutations artificially. In the breeding of new wheat varieties, as many as 264 new cultivars of wheat have been developed using physical mutagen gamma-ray irradiation, chemical mutagen Ethyl Methane Sulfonate (EMS), and fast neutrons (Jankowicz-Cieslak et al., 2016). However, the success of obtaining genetic diversity through mutation induction depends on the type of mutagen, the dose, and the radiosensitivity of the genetic material, but among these physical mutagens, gamma rays are more widely used (Khan et al., 2015; Wanga et al., 2020).

Recently, genetic improvement by using mutation has been one of the top priorities because more genetic variability is produced so that the required characters can be obtained quickly (Oladosu et al., 2014; Wanga et al., 2020). This study aimed to analyze the effect of various doses of gamma-ray irradiation on genetic variability in the agronomic character of several M2 populations derived from the sweet sorghum Super 2 mutant and to select potential populations for further selection programs.

MATERIAL AND METHODS

The research was carried out from February to June 2021 at the Cikeumeuh Experimental Garden, ICABIOGRAD, Bogor, Indonesia. The genetic material was the 2nd generation (M2) mutant population derived from *in-vitro* shoot irradiation of Super 2 variety with gamma rays at doses of 40, 50, 60, and 70 Gy. The control used was Super 2 variety.

Research Implementation

200 Mutant (M2) seeds from each radiation dose and control variety were planted at 75 cm between rows and 25 cm within. Each radiation dose number has five rows, and each row has 20 planting holes. Two seeds were planted per planting hole. Plant maintenance and fertilization are carried out according to the recommendations of sorghum cultivation. Harvesting is done when the seeds are physiologically ripe, marked by yellowing of the plant leaves, compact and perfect elliptical panicles, black spots at the base of the seeds, and hard seeds.

Observation

Observations were made on 50 samples from each dose of irradiation. The observed variables were: (1) plant height, (2) stem diameter, (3) panicle length, (4) fresh panicle weight, (5) dry panicle weight, and (6) sugar content (% Brix).

Data analysis

Statistical analysis is a ranking-based non-parametric data analysis because the data are not normally distributed and have high variability in treatment. It is because the M2 generation has a genetic constitution in the form of "Heterogeneous-Heterozygous" so it has high variability. A mean comparison between the treatment and the control and between doses of treatment was performed using a two-way t-test to see the differences between the observed characters. Furthermore, to see the differences between the doses of gamma irradiation mutagens, the Kruskal-Wallis test was carried out to determine whether there is a difference in the median between treatments.

Kruskal-Wallis test formula:

$$H = \frac{12}{N\,(N+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1)$$

$k = a$ number of samples;

ni = the number of cases in each sample i;

$N = \sum ni$ = the total number of cases;

R_i = total rank for each sample i;

$\sum ki = 1$ = shows the sum of all k samples (columns) approaching the Chi-square distribution with degree of freedom (df) = k-1 for a reasonably large sample size of n.

In addition to determining the appropriate selection method using a t-test, the value of genetic variance and heritability was also calculated. The estimation of broad sense heritability (h2bs) is calculated based on the formula (Singh and Chaudhary, 1979) as follows:

$$h^2bs = \sigma^2g/\sigma^2p$$

h^2bs = broad sense heritability

σ^2 p = phenotypic variance or M2 population variance

σ^2 e = environmental variance or control population

σ^2 g = genetic variance based on the formula (σ2 p - 2 e)

If the phenotypic variance is smaller than the environmental variance, the genetic variance is 0, or there is no genetic variability in the characters in the population.

The criteria for heritability values were determined based on Stansfield (1991), namely high ($h^2bs > 50\%$), medium ($20\% < h^2bs \leq 50\%$), and low ($h^2bs < 20\%$) categories. The value of the broadness or narrowness of genetic variability is estimated from the value of genotype variance, where the criteria for broad or narrow genetic variability is determined based on the criteria of Asghar and Mehdi (2010) with the following conditions: if $2\sigma^2g > \sigma\sigma^2g$, then the genetic variability is broad, whereas if $\sigma^2g < \sigma\sigma^2g$ then the genetic variability is narrow.

Clustering analysis was carried out to determine the degree of dissimilarity of phenotypes between doses which would produce a dendrogram using the Gower method based on the unweighted average linkage indicated by the cophenetic distance value. Spearman correlation analysis was carried out because the data were not normally distributed due to outliers, and to determine the closeness between variables' values.

RESULTS AND DISCUSSION

Mutant Population Selection

The t-test on parental plant populations compared to various irradiated populations can be seen in Tables 2 to 5. The target character for improvement in this sorghum breeding program is "fresh panicle weight". The two-way t-test was used to determine which population can be continued in the selection process. Determining the main character in the assembly of new varieties is essential (Oladosu et al., 2016).

The results of the t-test indicate variations in agronomic characters in the mutant population. Variations in fresh panicle weight character in the mutant population showed that the gamma-ray mutagen resulted in genetic variability in the M2 population, so better characters were obtained than before. The variation obtained shows that the mutation treatment can cause changes in certain characters without changing other characters (Khan et al., 2015). The role of mutations in increasing genetic diversity can be applied to plant genetic improvement

(Khursheed et al., 2018). Furthermore, to get superior characters, the right type of selection programs are significant (Khursheed et al., 2018).

Table 1. The t -value of control population (0 Gy) with M2 population (40 Gy)

Character	Control (0 GY)		M2 population (40 GY)		
	X + SD	Range	X + SD		Range
Plant height (cm)	308.02 ± 22.13	225 - 389	313.06 ± 19.74	ns	220 - 336
Bottom stem diameter (mm)	17.55 ± 1.87	14.5 - 24	17.78 ± 1.89	ns	14 - 22
Middle stem diameter (mm)	15.51 ± 1.87	12 - 22	15.62 ± 1.61	ns	11 - 19
Middle stem diameter (mm)	11. 68 ± 2.31	9 - 19	10.72 ± 1.30	*	8 - 14
Panicle length (cm)	25.62 ± 3.04	18 - 34	27.86 ± 4.52	**	16 - 45
Panicle diameter (mm)	45.65 ± 10.42	24 - 70	45.16 ± 12.15	ns	14 - 71
Sugar Brix content (%)	14.16 ± 2.33	9 - 18	15.16 ± 2.33	*	10 - 21
Wet panicle weight (g)	87.77 ± 27.28	49.23 - 164.44	88.81 ± 19.41	ns	36.54 - 148.95

Note: ns) not significant α = 0.05 based on the results of the t-test; *) significantly different α = 0.05 based on the results of the t-test; **) significantly different α = 0.05 based on the results of the t-test; SD: standard deviation.

In Table 1 the comparison between the parent population and the M2 population derived from 40 Gy irradiation showed significant differences in the characteristics of top stem diameter, panicle length, and sugar content. The results showed that the radiation treatment resulted in changes in agronomic characters and yields. In this study, the explants used were embryos because they consist of actively dividing tissue and also can avoid the occurrence of chimeras. The factors that influence the variation in mutants are the genotype used, the dose of irradiation, and the type of mutagen (Khursheed et al., 2018). The mutagen can produce changes directly or indirectly to genes that result in changes in their function and structure. General changes can be seen in plants' morphological, anatomical, biochemical, and physiological characteristics (Laskar, 2015).

The results of the t-test on the parental population compared to the M2 population derived from 50 Gy, showed that the characters were significantly different in panicle diameter, sugar content, and fresh panicle weight (Table 2).

Table 2. The t-value of control population (0 Gy) with M2 population (50 Gy)

Character	Control (0 GY)		M2 population (50 GY)		
	X + SD	Range	X + SD		Range
Plant height (cm)	308.02 ± 22.13	225 - 389	308.50 ± 18.52	ns	207 - 350
Bottom stem diameter (mm)	17.55 ± 1.87	14.5 - 24	17.86 ± 2.15	ns	14 - 23
Middle stem diameter (mm)	15.51 ± 1.87	12 - 22	15.88 ± 2.02	ns	12 - 20
Top stem diameter (mm)	11. 68 ± 2.31	9 - 19	11.80 ± 1.65	ns	9 - 15
Panicle length (cm)	25.62 ± 3.04	18 - 34	25.35 ± 2.74	ns	22 - 38
Panicle diameter (mm)	45.65 ± 10.42	24 - 70	38.48 ± 7.62	**	25 - 66
Sugar Brix content (%)	14.16 ± 2.33	9 - 18	17.83 ± 1.56	**	13 - 20
Wet panicle weight (g)	87.77 ± 27.28	49.23 - 164.44	61.97 ± 19.23	**	28.60 - 118.50

Note: ns) not significant α = 0.05 based on the results of the t-test; *) significantly different α = 0.05 based on the results of the t-test; **) significantly different α = 0.05 based on the results of the t-test; SD: standard deviation.

The results of the t-test of the parental population compared to the M2 population derived from 60 Gy showed different characteristics in plant height, top stem diameter, sugar content, and fresh panicle weight (Table 3).

Table 3. The t-value of control population (0 Gy) with M2 population (60 Gy)

Character	Control (0 GY)		M2 population (60 GY)		
	X + SD	Range	X + SD		Range
Plant height (cm)	308.02 ± 22.13	225 - 389	320.46 ± 15.31	**	286 - 360
Bottom stem diameter (mm)	17.55 ± 1.87	14.5 - 24	18.16 ± 2.43	ns	15 - 30
Middle stem diameter (mm)	15.51 ± 1.87	12 - 22	16.06 ± 2.75	ns	14 - 23
Middle stem diameter (mm)	11. 68 ± 2.31	9 - 19	12.96 ± 1.50	**	10 - 19
Panicle length (cm)	25.62 ± 3.04	18 - 34	24.90 ± 1.84	ns	20 - 32
Panicle diameter (mm)	45.65 ± 10.42	24 - 70	45.32 ± 8.14	ns	30 - 65
Sugar Brix content (%)	14.16 ± 2.33	9 - 18	15.76 ± 1.43	**	12 - 19
Wet panicle weight (g)	87.77 ± 27.28	49.23 - 164.44	71.70 ± 19.01	**	32.69 - 134.80

Note: ns) not significant α = 0.05 based on the results of the t-test; *) significantly different α = 0.05 based on the results of the t-test; **) significantly different α = 0.05 based on the results of the t-test; SD: standard deviation.

The results of the t-test of the parental population compared to the population of M2 derived from 70 Gy showed different characters only in the diameter of the bottom and middle stems (Table 4).

Table 4. The t value of control population (0 Gy) with M2 population (70 Gy)

Character	Control (0 GY)		M2 population (70 GY)		
	X + SD	Range	X + SD		X + SD
Plant height (cm)	308.02 ± 22.13	225 - 389	312.84 ± 19.72	ns	280 - 395
Bottom stem diameter (mm)	17.55 ± 1.87	14.5 - 24	19.21 ± 2.18	**	15.5 - 26
Middle stem diameter (mm)	15.51 ± 1.87	12 - 22	16.59 ± 2.00	**	13 - 21
Middle stem diameter (mm)	11. 68 ± 2.31	9 - 19	11.25 ± 1.74	ns	8 - 16
Panicle length (cm)	25.62 ± 3.04	18 - 34	26.62 ± 2.86	ns	15 - 30
Panicle diameter (mm)	45.65 ± 10.42	24 - 70	49.29 ± 9.83	ns	33.5 - 72
Sugar Brix content (%)	14.16 ± 2.33	9 - 18	13.88 ± 3.22	ns	9 - 23
Wet panicle weight (g)	87.77 ± 27.28	49.23 - 164.44	91.29 ± 29.21	ns	41.29 - 174.64

Note: ns) not significant α = 0.05 based on the results of the t-test; *) significantly different α = 0.05 based on the results of the t-test; **) significantly different α = 0.01 based on the results of the t-test; SD: standard deviation.

The t value presented in Table 1-4 shows that radiation dose treatment has a significant effect, as evidenced by genetic changes on agronomic characters in mutant populations. Changes in character produce genetic variability that can be selected to obtain populations with the required characters. The summary of the observed changes in agronomic characters can be seen in Table 5.

Table 5. Synthesis of the results of the t-test of the parental population and the M2 population at various irradiation doses

Character	Control Population (0 Gy)	M2 population							
		40 Gy		50 Gy		60 Gy		70 Gy	
Plant height (cm)	308.02	313.06	ns	308.50	ns	320.46	**	312.84	ns
Bottom stem diameter (mm)	17.55	17.78	ns	17.86	ns	18.16	ns	19.21	**
Middle stem diameter (mm)	15.51	15.62	ns	15.88	ns	16.06	ns	16.59	**
Top stem diameter (mm)	11. 68	10.72	*	11.8	ns	12.96	**	11.25	ns
Panicle length (cm)	25.62	27.86	**	25.35	ns	24.9	ns	26.62	ns
Panicle diameter (mm)	45.65	45.16	ns	38.48	**	45.32	ns	49.29	ns
Sugar Brix content (%)	14.16	15.16	*	17.83	**	15.76	**	13.88	ns
Wet panicle weight (g)	87.77	88.81	ns	61.97	**	71.70	**	91.29	ns

Note: ns) not significant α = 0.05 based on the results of the t-test; *) significantly different α = 0.05 based on the results of the t-test; **) significantly different α = 0.01 based on the results of the t-test; SD: standard deviation.

The differences between each character of M2 and their parents' populations were observed using the median value of the Kruskal-Wallis test. The fresh panicle weight character of the sorghum mutant population from a radiation dose of 70 Gy seemed to give the highest median value among other radiation treatments, namely 87.42 (Table 6). Therefore, to match the initial objectives of this sorghum breeding program, the main character chosen is "Fresh panicle weight". Characterization is one of the critical stages in a plant breeding program (Surahman et al., 2009). Characterization can be used as a basis for grouping plants (Anshori et al., 2019).

Table 6. Analysis of the difference in median values based on the Kruskal-Wallis test

Character	0 Gy	M2 Population				Nilai-H
		40 Gy	50 Gy	60 Gy	70 Gy	
Plant height (cm)	309.50	318.00	310.00	319.50	311.00	17.50 **
Bottom stem diameter (mm)	17.50	18.00	18.00	18.00	19.00	18.28 **
Middle stem diameter (mm)	15.00	16.00	16.00	16.00	16.50	10.95 *
Top stem diameter (mm)	11.25	10.50	12.00	13.00	11.00	46.83 **
Panicle length (cm)	25.00	27.00	24.75	25.00	27.00	39.30 **
Panicle diameter (mm)	44.00	45.50	38.00	45.00	47.00	34.90 **
Sugar Brix content (%)	14.50	15.00	18.00	16.00	13.00	76.93 **
Wet panicle weight (g)	84.43	84.27	58.18	71.65	87.42	57.06 **

Note: *) significantly different α = 0.05 based on the results of the t-test; **) significantly different α = 0.01 based on the results of the t-test.

The effect of radiation dose on the differences in character can be seen in the t-value between treatments (Table 7). The character of the sugar content in the stems of 70 Gy sorghum mutant population showed a t value that was not significantly different from the parents, but when compared to 40, 50, and 60 Gy, the t values were significantly different. Table 5, showed that at 70 Gy irradiation, the average sugar content of stems was 1% to 4% lower than those at the doses of 40, 50, and 60 Gy. The percentage of sugar content represents the total solid content which is positively correlated with the total sugar concentration in the sap of sweet sorghum (Davila-Gomez et al., 2011). Sap from sweet sorghum with a sugar content of >10% is sufficient to convert into sugar for ethanol production (Khalil et al., 2015; Kim and Day, 2011).

Table 7. t-value analysis between radiation dose treatments in the M2 generation of sweet sorghum

The M2 generation being compared Gy	t-count value							
	PH	BSD	MDS	TSD	PL	PD	SBC	WPW
0 vs 40	- 1.20 ns	- 0.61 ns	- 0.32 ns	2.56 *	- 2.91 **	0.22 ns	- 2.14 *	- 0.22 ns
0 vs 50	- 0.12 ns	- 0.77 ns	- 0.95 ns	- 0.30 ns	0.47 ns	3.93 **	- 9.25 **	5.39 **
0 vs 60	- 3.27 **	- 1.41 ns	- 1.56 ns	- 3.28 **	1.43 ns	0.18 ns	- 4.14 **	3.37 **
0 vs 70	- 1.14 ns	- 4.07 **	- 2.78 **	1.06 ns	- 1.69 ns	- 1.79 ns	0.50 ns	- 0.61 ns
40 vs 50	1.19 ns	- 0.20 ns	- 0.71 ns	- 3.63 **	3.36 **	3.29 **	- 6.73 **	6.95 **
40 vs 60	- 2.09 *	- 0.87 ns	- 1.35 ns	- 7.98 **	4.29 **	- 0.08 ns	- 1.55 ns	4.45 **
40 vs 70	0.06 ns	- 3.49 **	- 2.66 **	- 1.70 ns	1.63 ns	- 1.86 ns	2.27 *	- 0.50 ns
50 vs 60	- 3.52 **	- 0.65 ns	- 0.49 ns	- 3.68 **	0.96 ns	- 4.34 **	6.93 **	- 2.54 *
50 vs 70	- 1.13 ns	- 3.11 **	- 1.76 ns	1.63 ns	- 2.26 *	- 6.10 **	7.75 **	- 5.89 **
60 vs 70	2.15 *	- 2.27 *	- 1.44 ns	5.26 **	- 3.56 **	- 2.18 *	3.75 **	- 3.95 **

Note: ns) not significant α = 0.05 based on the results of the t-test; *) significantly different α = 0.05 based on the results of the t-test; **) significantly different α = 0.01 based on the results of the t test; SD: standard deviation;
PH: Plant height (cm); BSD: Bottom stem diameter (mm); MSD: Middle stem diameter (mm); TSD: Top stem diameter (mm); PL: Panicle length (cm); PD: Panicle diameter (mm); SBC: Sugar Brix content (%); WPW: Wet panicle weight (g).

Based on the t value between the M2 population on the target character, namely fresh panicle weight (FPW), two potential populations were obtained and could be continued in the selection process. They were the M2 population derived from irradiation doses of 40 Gy and 70 Gy. It can be seen from the 40 Gy mutant population, which shows a t value that is not significantly different from the control/parental population but shows a significant and positive t value when compared to the 50 Gy and 60 Gy populations. It was shown that the average fresh panicle weight of the 40 Gy M2 population was significantly higher than the two populations (Table 5).

Meanwhile, the 70 Gy mutant population also showed a value that was not significantly different from the control/parental population but showed a significantly different and positive t value when compared to the 50 Gy and 60 Gy populations.

It was shown that the average fresh panicle weight of the 70 Gy M2 population was significantly higher than the two populations (Table 5).

Table 8. Genetic variation and heritability of agronomic characters at various radiation dose

Karakter	Generasi M2	σ^2 p	σ^2 e	σ^2 g	2σ σ^2g	h^2 bs (%)
Plant height (cm)	40 Gy	389.81	489.78	0.00^S	0.00	0.00^R
	50 Gy	342.83		0.00^S	0.00	0.00^R
	60 Gy	234.25		0.00^S	0.00	0.00^R
	70 Gy	388.89		0.00^S	0.00	0.00^R
Bottom stem diameter (mm)	40 Gy	3.56	3.49	0.07^S	0.53	1.99^R
	50 Gy	4.61		1.12^S	2.12	24.30^M
	60 Gy	5.89		2.40^S	3.10	40.73^M
	70 Gy	4.77		1.28^S	2.26	26.81^M
Middle stem diameter (mm)	40 Gy	2.58	3.50	0.00^S	0.00	0.00^R
	50 Gy	4.07		0.57^S	1.51	14.06^R
	60 Gy	2.75		0.00^S	0.00	0.00^R
	70 Gy	4.01		0.51^S	1.43	12.78^R
Top stem diameter (mm)	40 Gy	1.70	5.36	0.00^S	0.00	0.00^R
	50 Gy	2.74		0.00^S	0.00	0.00^R
	60 Gy	2.24		0.00^S	0.00	0.00^R
	70 Gy	3.01		0.00^S	0.00	0.00^R
Panicle length (cm)	40 Gy	20.45	9.26	11.19^L	6.69	54.71^T
	50 Gy	7.48		0.00^S	0.00	0.00^R
	60 Gy	3.40		0.00^S	0.00	0.00^R
	70 Gy	8.17		0.00^S	0.00	0.00^R
Panicle diameter (mm)	40 Gy	147.68	108.62	39.06^L	12.50	26.45^M
	50 Gy	58.05		0.00^S	0.00	0.00^R
	60 Gy	66.18		0.00^S	0.00	0.00^R
	70 Gy	96.67		0.00^S	0.00	0.00^R
Sugar Brix content (%)	40 Gy	5.443	5.44	0.00^S	0.00	0.00^R
	50 Gy	2.425		0.00^S	0.00	0.00^R
	60 Gy	2.033		0.00^S	0.00	0.00^R
	70 Gy	10.36		4.92^L	4.43	47.46^M
Wet panicle weight (g)	40 Gy	376.63	744.14	0.00^S	0.00	0.00^R
	50 Gy	369.73		0.00^S	0.00	0.00^R
	60 Gy	361.30		0.00^S	0.00	0.00^R
	70 Gy	853.15		109.01^L	20.88	12.78^R

Note: T = high heritability, M = medium heritability, R = low heritability, L = wide genetic diversity.

Genetic Variance and Heritability Analysis

The genetic variance and heritability analysis were carried out to determine the appropriate selection method and the magnitude of the resulting selection progress. Heritability is a very important parameter in selection activities (Islam et al., 2015). The results of Anshori et al. (2019) show that if a heritability value is

above 60%, all characters have the potential to be used as selection characters. Based on the fresh panicle weight, the 70 Gy population of M2 was selected for further selection compared to the 40 Gy M2 population because it has a broad genetic variability. The heritability for fresh panicle weight in 70 Gy (12.78%) was also better than in 40 Gy (0.00%) (Table 8).

Selection in the M2 population (70 Gy)

An analysis of the M2 population (70 Gy) was carried out using Spearman correlation to determine the effect between the supportive characters and the main character (fresh panicle weight). The results of the Spearman correlation analysis showed that the characteristics of bottom stem diameter, middle stem diameter, top stem diameter, panicle length, and panicle diameter were significantly and positively correlated with the fresh panicle weight character so that increasing the value of these characteristics could increase the fresh panicle weight of sorghum by the coefficient value (Table 9).

Table 9. Correlation of quantitative characters in the M2 population of 70 Gy gamma-ray irradiation

	PH	BSD	MDS	TSD	PL	PD	SBC	WPW
PH	1							
BSD	-0.054 ns	1						
MSD	-0.031 ns	0.773**	1					
PL	-0.035 ns	0.783**	0.818**	1				
PL	0.031 ns	0.754**	0.798**	0.845**	1			
PD	-0.063 ns	0.244 ns	0.212 ns	0.429**	0.247 ns	1		
SBC	-0.196 ns	0.002 ns	0.012 ns	0.182 ns	0.007 ns	0.210 ns	1	
WPW	0.075 ns	0.414**	0.373**	0.504**	0.440**	0.447**	0.204 ns	1

Note: ns) not significant α = 0.05 based on the results of the t-test; *) significantly different α = 0.05 based on the results of the t-test; **) significantly different α = 0.01 based on the results of the t-test; SD: standard deviation;
PH: Plant height (cm); BSD: Bottom stem diameter (mm); MSD: Middle stem diameter (mm); TSD: Top stem diameter (mm); PL: Panicle length (cm); PD: Panicle diameter (mm); SBC: Sugar Brix content (%); WPW: Wet panicle weight (g).

Grouping of mutant genotypes based on cluster gram analysis

From the cluster gram analysis, 49 mutant genotypes from the 70 Gy M2 population were grouped into two main clusters (Figure 1). 35 genotypes were clustered in the first cluster together with the Super-2 variety, while the other 14 were clustered in the second cluster. The first group showed mutant genotypes that still had high similarity with the Super-2 variety. It can be seen from the color intensity that it tends to be evenly distributed in the cluster and the absence of varying colour intensity. The high similarity between the mutant genotypes in the first cluster and the Super-2 variety indicated that these genotypes did not experience many genetic changes due to the gamma-ray irradiation treatment.

Meanwhile, the genotypes in the second cluster showed low similarity to the Super-2 variety, which was seen from the color intensity that was more varied than the color seen in the first cluster. Genotype no. 30 has the highest degree of similarity with the Super-2 variety (92.7%), while genotype no. 35 has the lowest similarity level (63.9%). Clustergram analysis is an effective method for selection through grouping. This grouping aims to determine the relationship between plant genotypes and can be a material consideration in the selection process. Selection through grouping will be effective if the characteristics of the group can be known (Anshori et al., 2019). Clustergram analysis is a multivariate analysis that combines several cluster analyses in a flat dimension (Schonlau, 2002).

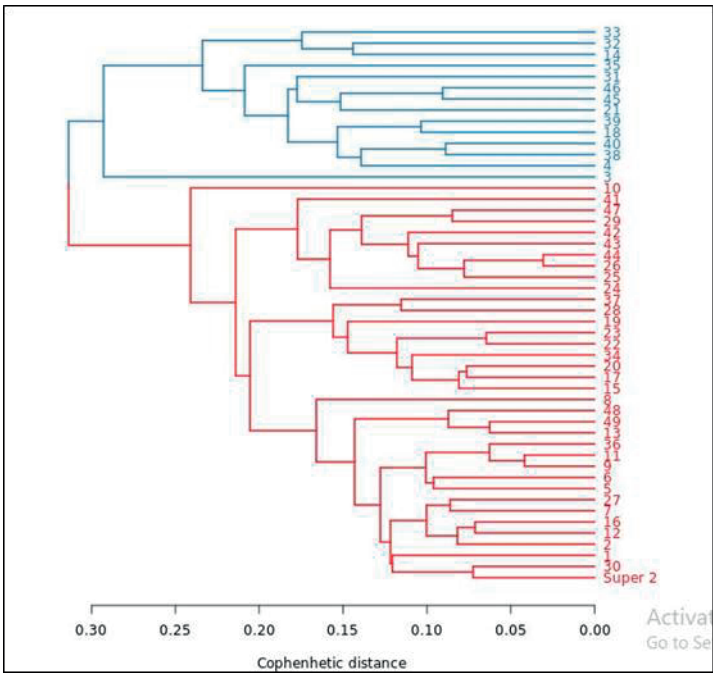

Figure 1. Dendrogram clusters on 49 sorghum Super-2 mutant genotypes

CONCLUSIONS

Gamma-ray irradiation with doses of 40, 50, 60, and 70 Gy can induce variations in the agronomic characters of M2 sweet sorghum mutants derived from the Super 2 sorghum variety. Significantly different responses of characters compared to their parents were seen in each radiation dose treatment.

Variation in characters was found in top stem diameter, panicle length, and sugar content for the 40 Gy M2 population. In the 50 Gy M2 population the differences were in panicle diameter, sugar Brix, and fresh panicle weight. In the 60 Gy M2 population, the differences were in plant height, top stem diameter, sugar content, and fresh panicle weight. In the 70 Gy M2 population, the differences were in the bottom and middle stem diameter. Based on the fresh weight character of the panicle, the 70 Gy M2 population could be continued for further selection. Pre-analysis using 49 mutant genotypes from the 70 Gy M2 population, resulted in two main clusters, where 35 genotypes were clustered together in the first cluster along with their original parent (Super-2 sorghum variety), while the other 14 were clustered in the second cluster.

AUTHOR CONTRIBUTION

All authors contributed equally to the manuscript as the main contributors. All authors conducted the research, observing and collecting the data and preparing the paper, i.e. performed the data analysis, discussed, interpreted the final results, and drafted the manuscript.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge ICABIOGRAD, who has provided experimental station facilities, and technicians, i.e. Mr Suparjo, Mr Agus Mashuri and Ms Ratna Utari, who has helped in plant preparation and yield processing

REFERENCES

Almodares, A., Hadi, M.R., Kharazian, Z.A., 2011. *Sweet sorghum: salt tolerance and high biomass sugar crop*. In: Matovic, D. (eds.), Biomass-Detection, Production, and Usage: 22-460.

Anshori, M.F., Purwoko, B.S., Dewi, I.S., Ardie, S.W., Suwarno, W.B., Safitri, H., 2019. *Selection index based on multivariate analysis for selecting doubled-haploid rice lines in lowland saline prone area*. Sabra Journal of Breeding and Genetics, 51(2): 161-174.

Arisha, M.H., Shah, S.N.N., Gong, Z.H., Jing, H., Li, C., Zhang, Hi-X., 2015. *Ethyl methane sulfonate induced mutations in M2 generation and physiological variations in M1 generation of peppers (Capsicum Annuum L.)*. Frontiers in Plant Science, 6: 1-11.

Asghar, M.J., and Mehdi, S.S., 2010. *Selection indices for yield and quality traits of sweet corn*. Pak. J. Bot., 42: 775-789.

Davila-Gomez, F.J., Chuck-Hernandez, C., Perez-Carrilloa, E., Rooney, W.L., Serna-Saldivar, S.O., 2011. *Evaluation of bioethanol production from five varieties of sweet and forage sorghums [Sorghum bicolor (L) Moench]*. Industrial Crops and Products, 33: 611-616.

Damardjati, D.S., Widowati, S., Wargiono, J., Purba, S., 2000. *Potential and utilization of local food resources cereals, tubers and nuts for food diversification*. Puslitbang Tanaman Pangan: 24-30.

Harmini, H., 2021. *Utilization of sorghum as ruminant feed in dry land*. Livest. Anim. Res., 19(2): 159-170.

Islam, M.A., Raffi, S.A., Hossain, M.A., Hasan, A.K., 2015. *Analysis of genetic variability, heritability and genetic advance for yield and yield associated traits in some. promising advanced lines of rice*. Progressive Agriculture, 26(1): 26-31.

Jankowicz-Cieslak, J., Tai, J.T., Kumlehn Till, B.J., 2016. *Biotechnologies for plant mutation breeding: protocols*. Biotechnologies for Plant Mutation Breeding: Protocols: 1-340.

Khalil, S.R.A., Abdelhafez, A.A., Amer, E.A.M., 2015. *Evaluation of bioethanol production from juice and bagasse of some sweet sorghum varieties*. Annals of Agricultural Science: 1-8.

Khan, M.A., Rakesh, Ch., Verma, R., 2015. *Assessment of the effects of gamma radiations on various morphological and agronomic traits of common wheat (Triticum Aestivum L.) Var. WH-147*. European Journal of Experimental Biology, 5(7): 6-11.

Khursheed, S., Raina, A., Laskar, R.A., Khan, S., 2018. *Effect of gamma radiation and EMS on mutation rate: their effectiveness and efficiency in faba Ban (Vicia Faba L.)*. Caryologia, 71(4): 397-404.

Kim, M., and Day, D.F., 2011. *Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills*. J. Ind. Microbiol. Biotechnol., 38: 803-807.

Laskar, R.A., Khan, S., Khursheed, S., Raina, A., Amin, R., 2015. *Quantitative analysis of Induced phenotypic diversity in chickpea using physical and chemical mutagenesis*. Journal of Agronomy, 14(3): 102-111.

Lestari, E.G., 2021. *Mutation induction application for ornamental plant breeding*. Berita Biologi, 20(3): 1-10.

Lestari, E.G., Dewi, I.S., Nur, A., Yunita, R., Mastur, 2019. *Genetic x environment interaction on agronomic characters and yield components of sweet sorghum (Sorghum Bicolor) mutant strain*. Biodiversitas, 20(12): 3705-3714.

Londra, I.M., and Sutami, P., 2018. *Management of the use of sweet sorghum for Bali cows*. J. Manajemen Agribis, 8(2): 189-195.

Nouri, H., and Tavakoli, A., 2012. *Effect of gamma rays on pod and seed production and Economic Yield in Pinto Bean Cultivar of Khomeini*. Annals of Biological Research, 3(5): 2399-2404.

Oladosu, Y., Rafii, M.Y., Abdullah, N., Malek, M.A., Rahim, H.A., Hussin, G., Latif, M.A., Kareem, I., 2014. *Genetic variability and selection criteria in rice mutant lines as revealed by quantitative traits*. Scientific World Journal, 2014: 1-14.

Oladosu, Y., Rafii, M.Y., Hussin, G., Ramli, N.A.A., Rahim, H.A., Miah, G., Usman, M., 2016. *Principle and application of plant mutagenesis in crop improvement: A review*. Biotechnology and Biotechnological Equipment, 30(1): 1-16.

Raina, A.R., Laskar, A., Khursheed, S., Khan, S., Parveen, K., Amin, R., Khan, R., 2017. *Induce physical and chemical mutagenesis for improvement of yield attributing traits and their correlation analysis in chickpea*. International Letters of Natural Sciences, 61: 14-22.

Raina, A., Khursheed, S., Khan, S., 2018. *Optimization of mutagen doses for gamma rays and sodium azide in cowpea*. Trends in Biosciences, 11(13): 2386-2389.

Rao, P.S., Kumar, C.G., Reddy, B.V.S., 2013. *Sweet sorghum: from theory to practice*. In: Rao, P.S., Kumar, C.G. (eds.), Characterization of improved sweet sorghum cultivars. Springer Briefs in Agriculture: 1-15.

Ratnavathi, C.V., Chakravarthy, K.S., Komala, V.V., Chavan, U.D., Patil, J.V., 2011. *Sweet sorghum as feedstock for biofuel production: a review*. Sugar Tech., 13(4): 399-407.

Reddy, B.V.S., Ramesh, S., Sanjana, R.P., Ramaiah, B., Salimath, P.M., Kachapur, R., 2005. *Sweet sorghum - A potential alternative raw material for bioethanol and bioenergy*. International Sorghum and Millets Newsletter, 46: 79-86.

Roychowdhury, R., and Tah, J., 2011. *Assessment of chemical mutagenic effects in mutation breeding programme for M1 generation of carnation (Dianthus Caryophyllus)*. Research in Plant Biology, 1(4): 23-32.

Schonlau, M., 2002. *The clustergram: a graph for visualizing hierarchical and nonhierarchical cluster analyses*. The Stata Journal: Promoting communications on statistics and Stata, 2(4): 391-402.

Singh, R.K., and Chaudary, B.D., 1979. *Biometrical methods in quantitative genetics analysis*. Kalyani Publisher, New Delhi.

Stansfield, W.D., 1991. *Theory and problem of genetics*. 3rd ed. Schaum's Outline Series, Mc Graw-Hill Inc., Singapore.

Suarni, S., 2012. *Potential of sorghum as a functional*

foodstuff. Iptek Tanaman Pangan, 7(1): 58-66.

Suarni, S., 2016. *The role of physicochemical properties of sorghum in food and industrial diversification and its development prospects*. Jurnal Litbang Pertanian, 35(3): 99-110.

Surahman, M., Santosa, E., Fifin, D., Nisy, N., 2009. *Molecular characterization and cluster analysis of Jatropha germplasms from Indonesia and other countries using morphological*. J. Agron. Indonesia, 37(3): 256-24.

Verma, A.K., Dhanasekar, P., Choudhary, S., Meena, R.D., Lal, G., 2018. *Estimation of induced variability in M2 generation of Fennel (Foeniculum Vulgare Mill)*. Pharmacognosy and Phytochemistry, 7(1): 430-436.

Wanga, M., Shimelis, A.H., Horn, L.N., Sarsu, F., 2020. *The effect of single and combined use of gamma radiation and ethyl methane sulfonate on early growth parameters in sorghum*. Plants, 9(7): 1-15.

Wirnas, D., Widodo, I., Sopandie, D., 2006. *Selection of agronomic characters to compile selection index in 11 soybean populations of generation F6*. Indonesian Journal of Agronomy, 34(1): 19-24.