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Research article

Genetic variability and heritability in 2nd generation mutant population from "Gando Keta" sorghum mutation

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Abstract

Importance of the work: The sorghum mutant "Gando Keta"is known to have a sticky texture but low production levels, with mutations expected to produce plants with improved yields.

Objectives: To use gamma radiation to produce sorghum mutants of "Gando Keta" with high yield and fluffy texture.

Materials & Methods: Explants (2 mm high, aged 3 d) were exposed to radiation doses in the range 10–100 Gy in 10 Gy increments. The shoots were acclimatized and their variability was observed. Then, 30 2nd generation mutant (M2) plants for each dose of 40 Gy, 50 Gy, 60 Gy and 70 Gy were planted and observed for plant height, panicle diameter, panicle length, wet and dry panicle weights and seed weight.

Results: Analysis of variance showed significant differences in mean plant height, stem diameter and panicle length. Radiation with doses of 40 Gy and 50 Gy produced significantly greater plant heights than those irradiated with a dose of 70 Gy. Observations on the growth characteristics of the six selected genotypes showed that the genotype "GK50-18" had a significantly greater plant height compared to M2, while the genotypes "GK60-13" and "GK70-5" were significantly lower than the M2 population. Seed weight had wide genetic variability and high heritability, so selection should be based on seed weight characters using the pedigree technique. Amylose analysis identified genotypes with lower amylose contents that should be further tested to determine the stability of the results.

Main finding: Selection on the 2nd generation mutant (M2) population obtained six genotypes with higher productivity: "GK50-18", "GK50-19", "GK60-11", "GK60-13", "GK60-9" and "GK70-5".

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Introduction

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important cereal crops in the world with uses as a human food source, animal feed, fodder and as a bioenergy raw material to produce biofuel and bioproducts (Ananda et al 2020). More than 500 million people in Asia and Africa rely on this cereal crop as their primary source of nutrition (Khalid et al., 2022). Along with other nations, Indonesia is currently experiencing difficulties due to food and energy crises. These issues might be resolved by using different regional food sources, such as sorghum (Pabendon et al., 2012). Sorghum has a high adaptability to unfavorable lands, such as arid areas and acidic soils, making it a cereal crop that can be produced under a variety of conditions, with the potential to be developed in Indonesia (Almodares and Hatamipour, 2011; Pabendon et al., 2012).

Sorghum has nutritional advantages over other cereals. For example, sorghum contains carbohydrates (about 70%) and a level of protein (8–12%) that is equivalent to wheat and higher than for rice (6–10%), as well as having a fat level (2–6%), higher than for rice (0.5–1.5%) and containing essential elements, such as P, Mg, Ca, Fe, Zn, Cu, Mn, Mo and Cr (Widowati, 2010; Suarni, 2012). As an alternative food, sorghum has good nutritional content, with even higher protein, calcium and vitamin B1 than rice and corn (Suarni, 2012).

Various bioactive substances, including phenolic acids, procyanidins, flavonoids and anthocyanins, are abundant in sorghum kernels (Xiong et al., 2019; Punia et al., 2020). These substances have been found to decrease oxidative stress and may have anti-cancer effects (Girard and Awika, 2018). Sorghum is an excellent food to lower the high prence of stunting because of its great nutritional value and low gluten level (Punia et al., 2020). According to Fitrahtunnisa and Rahmatullaila (2020), "Gando Keta" is a regional kind of sorghum that is utilized as an alternative food ingredient and is commonly processed into sorghum rice, porridge, pastries, tortillas and other snacks in Indonesia. Compared to other native Bima sorghums ("Gando Bura" and "Latu Kala"), "Gando Keta" has the highest antioxidant activity (Fitrahtunnisa et al., 2021).

High genetic variability is needed during plant breeding processes to produce the required traits (Wanga et al., 2018; Sushma et al., 2020). The nutritional quality of sorghum has improved through conventional plant breeding, biotechnology and crop management techniques, (Kumar et al., 2013). It is possible to increase plant genetic variety, particularly in "Gando Keta," by inducing mutagenesis using physical mutagens via gamma radiation (Raina et al., 2017).

The radiosensitivity of the genotype, which may be assessed by evaluating the lethal dose of gamma radiation necessary to prevent 50% of seed germination (LD_{50}), which determines the efficacy of gamma-ray irradiation. The LD_{50} value is calculated using the proportion of germinated seeds that were exposed to gamma radiation (Djarot et al., 2021). According to predictions made by Dwiatmini et al. (2009), an irradiation dose that falls inside the LD_{50} range can produce the most varied and numerous mutants.

Positive genetic alterations have been produced in several plants through the application of gamma-ray mutagenesis techniques (Lestari et al., 2016; Chaudhary and Deshmukh, 2019;

Anne and Lim, 2020; Lestari, 2021). Mutations have produced new and enhanced agronomic features relative to the wild types or parent plants, demonstrating promise in producing new plant varieties. For example, superior rice and sorghum cultivars have been created through the use of *in vitro* mutagenesis utilizing ^{60}Co in sorghum plant breeding (Lestari et al., 2019; Human et al., 2020; Yunita et al., 2020). The current research aimed to enhance the agronomic characteristics of the sorghum variety "Gando Keta," including yield and rice quality.

Materials and Methods

Study area and genetic material

The study was conducted from February 2021 to July 2022 at the Tissue Culture Laboratory of ICABIOGRAD (Indonesian Center for Research and Development of Biotechnology and Agricultural Genetic Resources) and Cikeumeuh Experimental Garden, Bogor. The genetic material used was the "Gando Keta" sorghum variety as the parent plant (wild type). The genetic material, "Gando Keta," has been registered at the Center for Plant Variety Protection and Agricultural Licensing, Ministry of the Republic of Indonesia, with the code 764/PVL/2018.

Procedures

The research procedures undertaken were: 1) induction of mutations in the explants of the original seed germinating *in vitro*; (2) evaluation of variability in the 1st generation mutants (M1); 3) variability, heritability and selection of 2nd generation mutants (M2); and 4) amylose content analysis.

Induction of mutations in seed germinating in vitro

Sorghum seeds of the "Gando Keta" variety were sterilized using a 10% and 20% Clorox solution for 20 and 5 minutes, respectively. Then, they were washed using sterile distilled water. The sterilized explants were planted on Murashige Skoog (MS) base medium without growth regulators (MS0) and stored at 21°C in a dark culture room.

At the Atomic Energy Agency's PAIR BATAN center (Center for Application of Radiation Isotopes), seeds that had been germinating for about 3 d were exposed to radiation at rates of 10–100 Gy in increments of 10 Gy. Each irradiation treatment dose utilized 100 seeds that had already begun to sprout were utilized. The germinated, irradiated seeds were transferred to an MS medium devoid of growth regulators to produce plantlets. Measurements were taken 7 d after planting to determine the percentage of shoot growth, shoot height and quantity of viable shoots.

Evaluation of agronomic character in 1st generation mutants

Plantlets aged 7 d were acclimated to a medium made from a 1:1 blend of soil and manure. The acclimated plants were placed in 5 kg polybags at age 4 wk. Watering, fertilizing and eradicating pests and plant diseases were all essential aspects of the plant care maintenance regime applied. The observed plants comprised 20 plants each from the treatment groups that received doses of 40 Gy, 50 Gy, 60 Gy and 70 Gy, in addition to the control group. Plant height (measured in centimeters), stem diameter (measured in millimeters), panicle length (measured in centimeters), fresh seed weight (measured in grams), and the percentage Brix were measured during harvest. Mutant plants from radiation dosages of 10 Gy, 20 Gy and 30 Gy were excluded from further consideration, because they did not exhibit any growth inhibition. The seeds of the 1st generation mutant (M1) plants were harvested based on radiation doses and stored separately for each plant.

Variability, heritability, and selection of 2nd generation mutants

In total, 31 M1 families from the previous generations were harvested and used for testing the 2nd generation mutants (M2). The test genotypes consisted of irradiated mutants at doses of 50 Gy (7 genotypes), 60 Gy (11 genotypes) and 70 Gy (13 genotypes). The genotypes obtained from 40 Gy irradiation were not considered further because their data did not show significant changes. In total, 30 plants were planted for each family, with a spacing of 75 cm between plant rows and 25 cm between rows. Fertilization was done twice: when the plants were aged 10 d and at 28 d after planting. Observations were conducted on each plantfor: plant height, stem diameter, panicle length, panicle diameter, wet panicle weight, dry panicle weight and seed weight. Analysis using a randomized complete block design was carried out on the differences between irradiation doses on mutant performance, as well as differences between M2 families (Table 4). Grouping was done based on replication, where replication was defined as the use of six plant samples as a repetition (six replicates). This was done to estimate the environmental variance, using the estimated mean square (EMS) according to the formula presented in Table 1.

Table 1 F test effects for randomized complete block experiments

Source of variation	Mean square	Expected mean square (fixed model)
Replication	M1	-
Genotype	M2	$\sigma^2e + r\sigma^2g$
Error	M3	σ^2e

σ^2e = environments variance; σ^2e = M3
σ^2g = genetic variance; = $\sigma^2g = M2 - M3/r$
σ^2p = phenotypic variance; = $\sigma^2 + \sigma^2e$
h^2bs = broad sense heritability; $h^2bs = \left(\frac{\sigma^2g}{\sigma^2p}\right) x100\%$
$\sigma(\sigma^2g)$ = standard deviation of genetic variance

Variability of amylose content

The analysis of the amylose and amylopectin contents was carried out on the 24 genotypes exposed to radiation doses of 50 Gy, 60 Gy and 70 Gy, as well as on a parent as the control. Testing was conducted at the Pakuan University Service Laboratory in Bogor, Indonesia. Difference analysis was used to determine the amylopectin concentration, while ultraviolet-visible spectrum spectrophotometry was used to determine the amylose content.

Data analysis

The quantitative data were analyzed using an F test with analysis of variance, followed by Duncan's multiple range test (DMRT). This analysis was performed using the SAS 9.4 software (SAS Intitute Inc., USA). Observational data were compared with the overall population mean using a t test based on a two-sample t test to compare the means of the two different populations. In addition, the data were analyzed based on Pearson's correlation coefficient (r) using

the Minitab-18.0 software (State Collage, PA, USA). The genetic parameters (variance and heritability) of M2 populations were examined and estimated using the Microsoft Excel 2010 software (Redmond, WA, USA), following the formula shown in Table 1.

Ethics statement

This study was approved by the Ethics Committee of the Research Center for Food Crops, National Research and Innovation Agency, Cibinong, West Java, Indonesia.

Results and Discussion

The observations of shoot growth in the explants treated with radiation indicated a decrease in growth viability with increasing irradiation doses (Table 2). In addition, there was a decrease in the ability of seeds to germinate at radiation treatment doses of 70–100 Gy reported by Djarot et al. (2021). The current results indicate that the mutagen dose for LD_{50} was about 86.74 Gy (producing a germination capacity of 50%). The LD_{50} dosage is the level that is expected to produce new characters in plant breeding through mutations so that selection can be carried out (Kurtar et al., 2017). The growth of seed germinating from the radiation treatments can be seen in Fig. 1, which shows that the higher the dose, the shorter the shoots produced.

Evaluation of variability in 1st generation mutants

Analysis of variance was performed in the M1 generation to determine the difference in the mean value of the mutant population in each radiation dose treatment. This analysis showed significant differences in mean values for plant height, stem diameter and panicle length. Plants irradiated with doses of 40 Gy and 50 Gy were significantly higher than those irradiated with a dose of 70 Gy. In contrast, plants irradiated at a dose of 70 Gy had a height that was not significantly different from the control population (0 Gy), as shown in Table 3. The panicle length of the control treatment plants and the irradiation dose of 60 Gy were significantly higher than the other doses. Plants irradiated with doses of 40 Gy and 60 Gy had a significantly higher average stem diameter than plants irradiated with a dose of 70 Gy. The results obtained indicated that the dose of irradiation given resulted in changes in the characteristics of plant height, panicle length and stem diameter. These results were consistent with the results of Putra et al. (2017), who reported that irradiation of tomato seeds at a dose of 100 Gy resulted in changes in the physiological growth curve of the plants, including leaf width, plant height, number of fruits and fresh fruit weight in the first week until harvest. Likewise, Arisha et al. (2015) obtained genetic variation in the M1 mutant of chili plants based on an EMS chemical mutagen treatment. Thus, mutation induction using physical or chemical mutagens has been shown to produce genetic variability in quality and quantity.

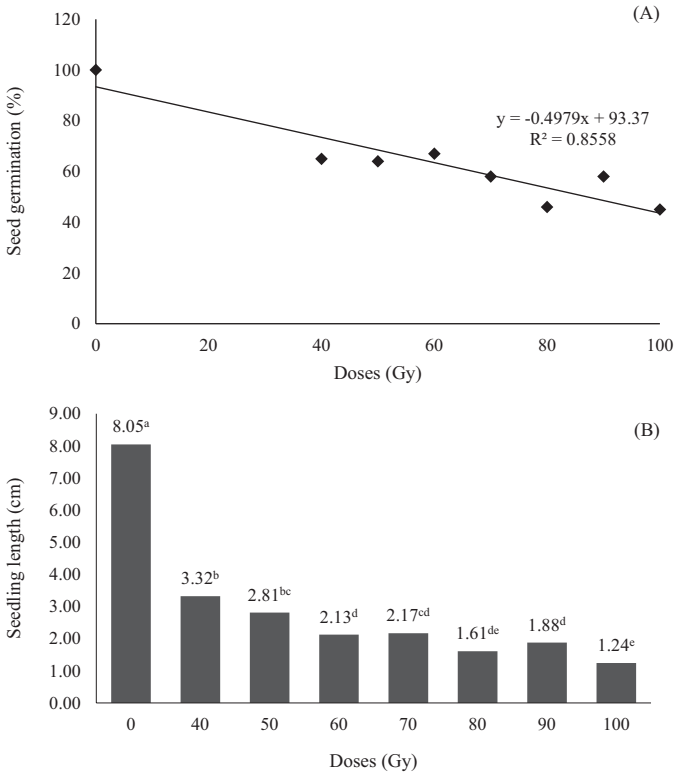

Fig. 1 Effect of different doses of irradiation treatment on: (A) germination; (B) sprout height (B), where chart columns with same lowercase letter are non-significantly ($p \geq 0.05$) different and R^2 = coefficient of determination.

Table 2 Germination and sprout height due to mutagen induction

Observed variable	Gamma ray irradiation mutagen dose (Gy)							
	0	40	50	60	70	80	90	100
Germination (%)	100	65	64	67	58	46	58	45
Sprout height (cm)	8.05	3.32	2.81	2.13	2.17	1.61	1.88	1.24

Table 3 Performance of plants resulting from gamma-ray irradiation in 1st generation mutants

Mutagenic dose (Gy)	Characters (Mean±standard deviation)				
	PH (cm)	PL (cm)	StemD (cm)	Brix (%)	SW (g/panicle)
0	222.85±15.16^{ab}	22.50±2.86^{a}	1.2±1.29^{ab}	12.28±3.38	49.47±17.87
40	228.75±18.97^{a}	18.39±2.04^{b}	1.2±1.00^{a}	13.82±2.53	34.83±14.00
50	230.00±18.78^{a}	19.28±3.00^{b}	1.1±0.98^{ab}	12.58±3.34	34.63±20.15
60	225.67±15.71^{ab}	22.00±2.35^{a}	1.2±1.87^{a}	8.70±2.82	34.12±18.34
70	214.68±24.29^{b}	18.50±2.61^{b}	1.1±1.35^{b}	12.58±3.28	34.1±15.78
Mean	224.39	20.13	1.2	11.99	38.79
F value	2.62*	6.33**	2.99*	2.41^{ns}	2.74^{ns}
Coefficient of variation (%)	6.93	12.83	9.99	23.94	49.83

PH = plant height (cm); PL = panicle length (cm); StemD = stem diameter (cm); SW = seed weight (g). Mean±SD in each column superscripted by different lowercase letters are significantly ($p < 0.05$) different; ns = non-significantly ($p \geq 0.05$) different; * = significantly different ($p < 0.0\ 5$); ** = highly significantly different ($p < 0.01$)

The current study used *in vitro* sprouts as material for radiation treatment. The resulting variations in M1 and M2 showed that the radiation doses resulted in changes in morphological and agronomic characters.

Most of the research on food crops aims to improve the quality and quantity of crop yields. The current study focused on improving yield, specifically seed weight (SW), for use in selecting potential mutants. In addition, a high amylopectin content is a goal of such breeding.

The analysis of variance regarding the radiation dose treatment indicated that radiation caused changes in plant morphology. However, the average seed weight was not significantly different between treatments. This could have been due to the relatively high variation in each radiation dose treatment, as indicated by their coefficient of variation (CV) values. The CV of seed weight had a fairly high value (49.83%), indicating there was considerable variation within each irradiated population.

From these results, an individual evaluation was carried out on each plant in the M1 mutant population to quantify the variation in seed weight (data not shown). From this evaluation, 31 families derived from four different doses were selected and used for evaluation of the next generation (M2).

Evaluation of variability, heritability and selection of 2nd generation mutants

Seed weight was used as the main character to determine productivity. In many plants, seed characters are used to select the best genotypes to breed. According to Meriaty et al. (2021), the number of pods characterizes a rather high genetic advancement, suggesting that there is a good chance that these traits will be passed down to the following generation and that selecting soybean genotypes with numerous pods is quite simple. With this knowledge, seed size, which is likewise correlated with productivity, becomes a useful selection criteria.

In this study, 31 families were evaluated individually to determine any differences in productivity between mutant families. In total, six plants were grown as biological replicates and observed for each selected mutant families. The results of the analysis of variance showed that there were no differences between replicates; however, there were differences between the tested mutant genotypes (Table 4). The mean analysis using DMRT in the M2 population showed that the "GK70-5" family had the highest average seed weight compared to the other genotypes. Five mutant families, ("GK50-18", "GK50-19", "GK60-11", "GK60-13" and "GK60-9") had average seed weights that were not significantly different from the "GK70-5" family. These genotypes and the "GK70-5" family were then selected and compared with the entire M2 population.

Observations on the growth characteristics of the six selected families showed that the "GK50-18" family had values that were significantly higher than for the M2 population, while the "GK60-13" and "GK70-5" families had values that were were significantly lower than the M2 population (Table 5). The stem diameter of all selected populations was not significantly different from that of the M2 population. The panicle length of the family "GK60-13" was significantly longer than for the M2 population. The "GK60-13" family also had a significantly larger panicle diameter than the M2 population, while for the other families, it was not significantly different.

The results from the panicle and seed observation showed that the family "GK60-13" had a significantly higher wet panicle weight than the M2 population. The families "GK60-13" and "GK70-5" had significantly higher dry panicle weights than the M2 population, with only the "GK60-9" family having seed weights that were not significantly different from the M2 population.

Table 4 Population of 2nd mutant generation resulting from gamma-ray irradiation

Population	Characters (mean ± standard deviation)						
	PH (cm)	StemD (cm)	PL (cm)	DP (mm)	WP (g)	DPW (g)	SW (g)
GK 50-17	253.55±13.58$^{b–h}$	1.18±0.14^{bcd}	17.04±2.27^{bc}	3.91±0.51$^{g–k}$	44.25±7.47$^{d–h}$	38.78±8.05$^{d–i}$	33.83±6.94$^{d–j}$
GK 50-18	261.03±8.39$^{a–d}$	1.24±0.12^{bcd}	18.69±1.03^{bc}	4.64±0.51$^{a–e}$	58.52±12.91^{abc}	53.02±11.95^{ab}	46.65±10.95^{ab}
GK 50-19	241.92±8.10^{d}	1.17±0.13$^{b–e}$	17.03±0.64^{bc}	4.37±0.43$^{a–i}$	51.45±6.25$^{a–e}$	46.07±5.68$^{b–f}$	39.72±4.75$^{a–f}$
GK 50-20	244.83±5.57$^{c–i}$	0.97±0.11^{e}	14.79±0.98^{c}	3.79±0.18^{ijk}	34.15±6.32^{ghi}	19.50±4.06^{k}	15.60±3.62^{l}
GK 50-7	271.35±3.74^{ab}	1.17±0.12$^{b–e}$	15.37±0.81^{c}	4.10±0.39$^{d–j}$	37.73±8.65$^{f–i}$	34.62±8.29$^{f–j}$	30.30±8.30$^{f–k}$
GK 50-8	273.55±21.52^{a}	1.13±0.13^{de}	17.05±1.24^{bc}	4.44±0.30$^{a–h}$	41.57±4.51$^{e–i}$	37.00±4.55$^{e–j}$	30.62±4.75$^{f–k}$
GK 50-9	259.08±13.56$^{a–e}$	1.30±0.16$^{a–d}$	16.25±2.66^{bc}	3.46±0.30^{k}	30.27±9.97^{j}	25.42±9.88^{jk}	21.02±9.66^{kl}
GK 60-1	247.00±15.26$^{c–l}$	1.18±0.12^{bcd}	17.68±0.83^{bc}	3.86±0.40$^{h–k}$	41.22±10.31$^{e–i}$	37.10±9.08$^{e–d}$	32.03±8.19$^{e–j}$
GK 60-10	235.67±10.86^{hij}	1.25±0.15^{bcd}	18.05±1.40^{bc}	3.69±0.64^{jk}	21.80±10.37^{j}	28.42±12.76^{ijk}	23.60±11.67^{jkl}
GK 60-11	258.67±15.37$^{a–f}$	1.25±0.19^{bcd}	18.37±1.18^{bc}	4.14±0.76$^{c–j}$	56.18±11.00$^{a–d}$	51.35±11.97^{abc}	45.77±9.94^{abc}
GK 60-13	198.93±8.18^{k}	1.24±0.09^{bcd}	20.18±1.25^{ab}	4.85±0.52^{a}	61.82±10.06^{ab}	51.10±4.29$^{a–d}$	43.37±3.36$^{a–d}$
GK 60-17	245.73±18.19$^{c–i}$	1.28±0.13$^{a–d}$	17.55±1.62^{bc}	3.91±0.422$^{g–k}$	44.43±9.42$^{d–h}$	41.57±9.14$^{b–g}$	34.22±8.52$^{d–j}$
GK 60-3	251.50±6.09$^{c–h}$	1.25±0.23^{bcd}	16.67±0.89^{bc}	4.21±0.44$^{b–j}$	47.93±6.23$^{c–f}$	39.97±6.64$^{c–i}$	35.82±5.14$^{b–i}$
GK 60-4	261.67±11.47$^{a–d}$	1.21±0.17^{bcd}	16.68±1.05^{bc}	4.60±0.36$^{a–f}$	47.78±7.61$^{c–f}$	40.85±6.19$^{b–h}$	34.97±5.74$^{c–i}$
GK 60-6	252.17±11.32$^{b–h}$	1.14±0.10^{cde}	16.89±1.19^{bc}	3.98±0.19$^{f–k}$	47.32±7.78$^{c–g}$	42.47±7.16$^{b–g}$	36.97±6.79$^{b–i}$
GK 60-7	236.13±13.79$^{g–j}$	1.18±0.11^{bcd}	17.32±1.27^{bc}	4.05±0.30$^{e–k}$	33.90±4.91^{hi}	29.13±6.53$^{h–k}$	26.37±5.57$^{h–k}$
GK 60-8	253.38±11.07$^{b–h}$	1.16±0.09$^{b–e}$	18.10±0.82^{bc}	4.13±0.44$^{c–j}$	50.07±5.78$^{b–f}$	45.25±3.53$^{b–f}$	37.97±3.95$^{b–g}$
GK 60-9	253.33±17.34$^{b–h}$	1.20±0.11^{bcd}	16.55±1.85^{bc}	4.12±0.76$^{c–j}$	57.34±16.12$^{a–d}$	49.07±13.49$^{a–e}$	43.28±11.43$^{a–e}$
GK 70-11	255.55±9.45$^{a–g}$	1.33±0.18$^{a–d}$	17.13±1.46^{bc}	4.71±0.61$^{a–d}$	44.32±11.99$^{d–h}$	41.10±11.24$^{b–h}$	33.82±10.22$^{d–j}$
GK 70-12	239.05±18.23$^{f–j}$	1.38±0.09^{ab}	18.13±1.61^{bc}	4.43±0.47$^{a–h}$	49.75±14.51$^{b–f}$	49.28±13.82$^{a–e}$	35.88±11.49$^{b–i}$
GK 70-13	169.08±12.21^{l}	1.39±0.16^{ab}	16.90±1.58^{bc}	4.47±0.46$^{a–h}$	47.97±10.56$^{c–f}$	40.80±8.45$^{b–h}$	34.55±8.58$^{d–j}$
GK 70-14	222.60±15.34^{j}	1.30±0.16$^{a–d}$	23.43±1.45^{a}	4.61±0.29$^{a–e}$	45.62±6.62$^{c–h}$	42.65±6.30$^{b–g}$	34.68±6.13$^{d–j}$
GK 70-15	262.88±15.13^{abc}	1.39±0.09^{ab}	18.17±1.64^{bc}	4.74±0.41^{abc}	51.45±6.15$^{a–e}$	45.82±4.73$^{b–f}$	37.55±4.72$^{b–h}$
GK 70-17	252.80±10.43$^{b–h}$	1.23±0.09^{bcd}	17.28±1.49^{bc}	4.54±0.63$^{a–g}$	34.35±9.89^{gh}	32.33±7.51$^{g–j}$	26.98±7.46$^{g–k}$
GK 70-18	254.87±14.71$^{a–h}$	1.19±0.16^{bcd}	17.68±1.50^{bc}	4.82±0.29^{ab}	42.08±10.82$^{e–i}$	36.55±9.47$^{f–j}$	27.50±9.22$^{g–k}$
GK 70-20	263.03±17.32^{abc}	1.47±0.44^{a}	17.82±1.32^{bc}	4.53±0.27$^{a–g}$	49.67±8.31$^{b–f}$	46.53±7.40$^{b–f}$	39.08±7.13$^{b–f}$
GK 70-3	243.93±10.18$^{c–i}$	1.28±0.09$^{a–d}$	16.60±1.32^{bc}	4.33±0.43$^{a–i}$	42.50±9.10$^{e–i}$	39.83±8.79$^{c–i}$	34.42±7.84$^{d–j}$
GK 70-5	231.22±10.87^{ij}	1.29±0.17$^{a–d}$	19.73±2.64^{b}	4.57±0.31$^{a–f}$	63.05±13.97^{a}	59.28±14.63^{a}	50.02±13.63^{a}
GK 70-7	242.45±15.8$^{d–i}$	1.36±0.10^{abc}	17.03±1.11^{bc}	4.05±0.30$^{e–k}$	33.54±4.78^{hi}	32.18±4.76$^{g–d}$	25.98±4.30^{ijk}
GK 70-8	239.93±18.3$^{e–j}$	1.26±0.11$^{a–d}$	17.85±0.58^{bc}	4.15±0.20$^{c–j}$	41.83±6.79$^{e–i}$	38.95±6.40$^{d–i}$	31.28±7.46$^{f–k}$
GK 70-9	244.33±20.79$^{c–i}$	1.32±0.08$^{a–d}$	17.15±0.65^{bc}	4.22±0.30$^{b–j}$	38.47±9.01$^{e–i}$	36.13±6.94$^{f–j}$	30.37±8.00$^{f–k}$
Grand mean	245.86±20.38	1.25±0.10	17.62±1.52	4.28±0.35	45.03±9.38	40.62±8.56	34.21±6.19
Population F value	12.81**	2.17**	1.55*	3.68**	5.82**	5.30**	4.92**
Replication F value	0.19^{ns}	0.55^{ns}	0.50^{ns}	1.11^{ns}	1.17ns	1.25^{ns}	1.70^{ns}
Coefficient of variation (%)	5.67	12.39	16.71	10.33	20.96	21.5	23.36

PH = plant height; StemD = stem diameter; PL = panicle length; DP = diameter of panicle; WP = wet panicle weight; DPW = dry panicle weight; SW = seed weight; GK = Gando Keta, 50, 60 and 70 indicate 50 Gy, 60 Gy and 70 Gy gamma doses respectively, and hyphenated trailing numbers Means in each column superscripted by different lowercase letters are significantly ($p < 0.05$) different; ns = non-significantly ($p \geq 0.05$) different; * = significantly different ($p < 0.05$); **= highly significantly different ($p < 0.01$)

Table 5 Population of 2nd mutant generation resulting from gamma-ray irradiation

Population	*n*	Characters (mean + standard deviation)			
		PH (cm)	StemD (cm)	PL (cm)	PD (mm)
GK 50-18	6	261.03±8.39**	1.235±0.121^{ns}	18.69±1.03^{ns}	4.640±0.511^{ns}
GK 50-19	6	241.92±8.10^{ns}	1.165±0.128^{ns}	17.03±0.64^{ns}	4.372±0.434^{ns}
GK 60-11	6	258.70±15.40^{ns}	1.247±0.187^{ns}	18.37±1.18^{ns}	4.138±0.760^{ns}
GK 60-13	6	198.93±8.18**	1.235±0.085^{ns}	20.18±1.25**	4.847±0.523*
GK 60-9	6	253.30±17.30^{ns}	1.203±0.110^{ns}	16.54±1.85^{ns}	4.123±0.531^{ns}
GK 70-5	6	231.20±10.90*	1.287±0.165^{ns}	19.73±2.64^{ns}	4.573±0.314^{ns}
Generation M2	184	245.90±23.80	1.250±0.168	17.62±3.05	4.276±0.531

PH= plant height; StemD = stem diameter (× 3.142); PL = panicle length; PD = panicle diameter; GK = Gando Keta, 50, 60 and 70 indicate 50 Gy, 60 Gy and 70 Gy gamma doses respectively, and hyphenated trailing numbers ns = non-significantly ($p \geq 0.05$) different based on an independent two-way t test; * = significantly ($p < 0.05$) different based on an independent two-way t test; ** = highly significantly ($p < 0.01$) different based on an independent two-way t test.

In this study, the M2 generation is the offspring of M1, so it is almost impossible for both generations to be grown at the same time (in the case of mutation, the diversity is random and cannot be duplicated). In addition, environmental variability has been estimated using a comparison in the form of elders (pure strains) grown using repetition. Therefore, the diversity observed in M1 and M2 is assumed to be diversity caused by genetic factors, so that the two populations can be compared, even though they were planted in different seasons. The selected genotypes were also compared with the M1 generation, where the results showed that the populations "GK50-18", "GK50-19", "GK60-11" and "GK60-9" had significantly higher plant height than the control population. Genotype "GK60-13" is known to have a significantly higher stem diameter than the control population of the M1 generation. All selected populations except "GK70-5" had significantly lower panicle lengths than the M1 generation control population. The seed weights of the selected genotypes were not significantly different from those of the M1 generation control population. Genotype "GK70-5" had values for mean plant height, stem diameter, panicle length and seed weight that were not significantly different from the control population of the M1 generation. These results indicated that the irradiation treatment given produced diversity in the observed variables, so that further selection could be carried out.

A component analysis of genetic variance and heritability was performed on the M2 population. The results showed that the stem diameter, panicle length and panicle diameter have narrow genetic diversity, so the potential for increasing these variables through selection in the M2 population would be relatively low. The seed weight variables had wide genetic diversity and high heritability, so selection for seed weight variables in M2 populations can be carried out (Table 6). Genetic variability and heritability are absolute requirements for the success of a plant breeding program (Sushma et al., 2020). High genetic variability will increase the opportunity to obtain superior characteristics that are not obtained in the parent or the wild type (Halide and Paserang, 2020).

Selection is essential in plant breeding activities (Raina et al., 2016; Yunandra et al., 2017). Seed character and productivity are the target characteristics in food plant breeding programs. As is the case with sorghum plants, in soybean plants, the number of filled pods can be used as a character for selection (Lestari et al., 2022). In the current study, seed weight (one of the seed characters) was used to select desirable sorghum genotypes.

One of the selection methods that can be used for population conditions with broad genetic variability and high heritability is the pedigree method. Pedigree selection is based on individual performances from the best families (Arifiana and Sjamsijah, 2017). This selection can be started in the early generations and is generally always recorded during the selection process, so the lineage is known in detail (Meriaty et al., 2021). This selection method also can be used for gaining a high yield sorghum variety.

Variability of amylose content

The amylose content was analyzed on the irradiated M2 mutants at doses of 50 Gy, 60 Gy, and 70 Gy, respectively. The analysis showed that the amylose content was in the range 23.01–37.01%, with an average of 30%, whereas the amylose content of the parent (wild type) genotype "Gando Keta" was 33.13% (Fig 2). The amylopectin content of sorghum seeds in this study was in the range 62.99–76.99%, with an average of 70%. Genotype "GK50-20" had the lowest amylose content, while "GK60-4" had the highest amylose content, 23.38% higher than the average.

Table 6 Genetic variance and heritability per character from M2 generation population

Character	σ^2e	σ^2g	σ^2p	$2\sigma(\sigma^2g)$	h^2bs (%)
Plant height	32.415	382.756W	415.171	39.128	92.192H
Stem diameter	0.004	0.005N	0.009	0.137	53.846H
Panicle length	1.443	0.788N	2.230	1.775	35.309M
Panicle diameter	0.033	0.087N	0.120	0.590	72.841H
Wet panicle weight	14.844	71.494W	86.338	16.911	82.807H
Dry panicle weight	12.713	54.709W	67.422	14.793	81.144H
Seed weight	10.644	41.768W	52.411	12.926	79.692H

H = high heritability; M = medium heritability; W = wide genetic diversity; N = narrow genetic diversity; h^2bs = broad sense heritability; σ^2e = environments variance; σ^2p = phenotypic variance, σ^2g= genetic variance or mutant variance, $\sigma(\sigma^2g)$ = standard error of genetic variance.
Note: heritability (%) based on Whirter (1979) is: low ($0 < X < 20\%$), moderate ($20 \leq X < 50\%$) and high ($50 \leq X$).

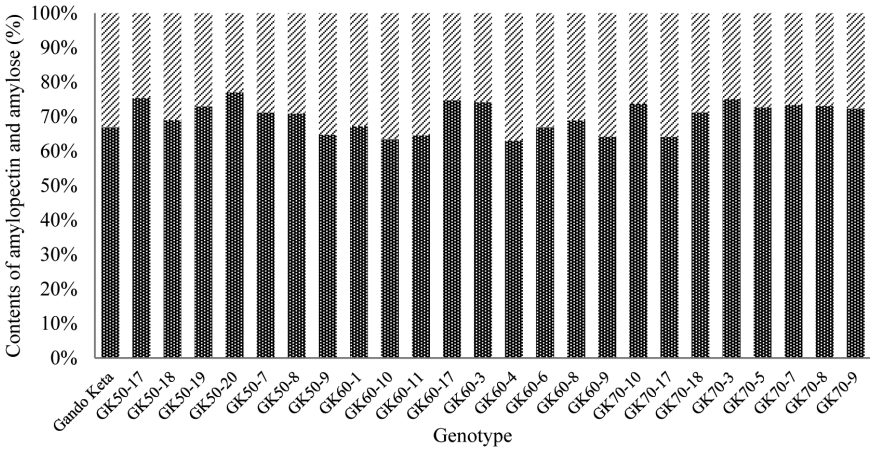

Fig. 2 Amylopectin and amylose contents of mutant genotypes, where GK = Gando Keta, 50, 60 and 70 indicate 50 Gy, 60 Gy and 70 Gy gamma doses respectively, and hyphenated trailing numbers

The amylose content of the 70 Gy-irradiated genotype was significantly lower than that of the 60 Gy-irradiated genotype based on an independent two-way t test (-2.39). Most of the genotypes irradiated at a dose of 70 Gy had amylose contents below the average, except for "GK70-17". In this study, the radiation treatment tended not to reduce the amylose content significantly.

An increase in radiation dose from 40 Gy to 70 Gy on sorghum variety Suri 3 produced a reported increase in the fat, protein, ash and amylopectin contents (Djarot et al., 2021). In the current study, analysis of the amylose content of several mutants showed that the mutant genotypes tended to have lower amylose content than their parents (wild type), so further tests are needed to determine the stability of these results. According to Suarni (2016), the amylose content in sorghum seeds will determine the texture of sorghum rice, with seeds with a lower amylose content tending to have a fluffier rice taste.

Stickiness is always negatively correlated with amylose content and hardness; thus, rice with a high amylose content is usually more rigid and less sticky. In contrast, rice with a low amylose content is softer and stickier. Nevertheless, rice cultivars with similar amylose contents can still display different stickiness (Ayabe et al., 2009), with classification into waxy (glutinous), low and intermediate (Tuaño et al., 2016). Seed weight has broad genetic variability and high heritability, so selection is made based on the character of seed weight using the pedigree technique. The current analysis of amylose in several of the mutants obtained genotypes with lower amylose content so that they could be tested further to determine the stability of the results.

From the results of evaluating two generations of mutants induced by gamma irradiation, two potential mutant genotypes were obtained with good agronomic characteristics, better productivity and a lower amylose content than the wild type. These two genotypes were "GK70-5" and "GK50-19"; they can be used as candidates for derived varieties with better seed quantity and quality than the original varieties. To strengthen this selection process, evaluating and purifying the selected genotypes in the next generation is necessary. The current results have shown that gamma radiation can produce better characters than their parents have, namely good agronomic characteristics, better productivity and a lower amylose content than the wild type. The current results should be very useful as material for further testing and selection.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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