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Gamma Radiation Effect on Growth, Production and Lignin Content of *Sorghum sudanense* at Different Harvest Ages

Maudi Nayanda Delastra, Andriyani Astuti, Bambang Suwignyo, Muhlisin and Nafiatul Umami*

Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada Yogyakarta, 55281, Indonesia

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* Corresponding author:
E-mail: nafiatul.umami@ugm.ac.id

ABSTRACT

The purpose of this study was to determine the effect of gamma-ray irradiation on *Sorghum sudanense* in the first offspring (F1) on plant growth, production, and lignin content at different harvest *ages*. This study used a split-plot design in which varieties of *Sorghum sudanense* with gamma irradiation and *Sorghum sudanense* without gamma irradiation are the main plot. Meanwhile, the harvest *age* is the subplot. The planting area was 1.5x1.5 m, each with 3 replications. The materials used were *Sorghum sudanense* without gamma-ray irradiation and the first generation seeds (F1) of *Sorghum sudanense* with gamma irradiation. The method used was irradiating *Sorghum sudanense* seeds with gamma-ray with a dose of 300 Gy, planting, maintenance, and harvesting. The harvest *ages* were 50, 70, and 90 days. The data observed were plant growth, namely plant height and length, dry matter (DM) and organic matter (OM) production, and lignin content. *Sorghum sudanense* with gamma irradiation had higher plant height, plant length, also DM, and OM production ($P<0.05$) than *Sorghum sudanense* without gamma irradiation. The lignin content of *Sorghum sudanense* with gamma irradiation was lower ($P<0.05$) than *Sorghum sudanense* without gamma irradiation. Longer harvest *age* increased ($P<0.05$) plant height, plant length, production, and lignin content. In conclusion, there were characteristics differences between *Sorghum sudanense* with gamma irradiation and without gamma irradiation (parents). The longer harvest led to higher plant height, length, production, and lignin content. There was an interaction ($P<0.05$) between *varieties and harvest ages*. Gamma irradiated *Sorghum sudanense* had a peak production at the age of 70 days, with a lignin content of 3.63%.

Keywords: Gamma-ray irradiation, Growth, Harvest *age*, Lignin, *Sorghum sudanense*,

Introduction

Sorghum is included in tropical cereal crops but can be grown in a wide climatic range. Godoy and Tesso (2013) stated that this plant has fairly good adaptability to marginal land conditions and is a native plant from tropical and subtropical regions in the southeastern Pacific and Australasia, an area which includes Australia, New Zealand, and Papua. Hence, this plant is suitable for Indonesia's climate.

One type of sorghum that has the potential to be used as animal feed is *Sorghum sudanense*. *Sorghum sudanense* is very likely to be an alternative source of forage plants in dry land. As a cut grass, this sorghum can grow back after being harvested which is better than other short-lived grasses (Wardhani, 1995). To accomplish sorghum as an alternative feed other than grass, abundant production is needed hence met the forage requirement as animal feed in Indonesia, especially for smallholder livestock. *Sorghum sudanense* has several advantages, but still has some disadvantages such as high lignin content.

Efforts to produce sorghum plants with superior varieties need to be carried out with methods or works to improve the genetic varieties of these plants. One way to improve genetic quality is to carry out plant breeding with a genetic mutation process. Henuhili and Sunarsih (2003) stated that grass breeding aims to collect superior properties of grass to serve as future superior grass. The breeding process through mutations can be done through artificial mutations. One of the ways is by physical mutation using gamma-ray irradiation. A mutation is the easiest method to get genetic diversity compared to other breeding methods. Mutation can change several characters, such as getting new traits and having superior traits that are not owned by the parent plant (Parry *et al*., 2009). The mutation with gamma-ray irradiation was chosen because it does not cause side effects on the mutant product, so it is safe for human consumption or livestock.

The process of engineering mutation plants with gamma irradiation requires the proper dose. Giving a too high dose will lead to cell division inhibition, cell death, decreasing plant growth process, growth power, and morphology. However, a radiation dose that is too low is not enough to mutate plants because the mutation frequency is too low and only produces a few mutated sectors (Hameed *et al*., 2008). The gamma-ray irradiation dose range in sorghum plants varies from 100 to 400 Gray. Surya and Soeranto (2006) stated that the highest diversity in sweet sorghum was between 100 to 300 Gray doses. Thereby, this study used a 300 Gray dose.

The effects of gamma-ray radiation can lead to both positive and negative properties. The properties that arise after gamma-ray irradiation are expected to increase the genetic diversity of the sorghum plant. It is also expected to produce good plant growth, high production, and lower lignin content. The traits that appear are heritable, but the genetic traits are often not the same for the generation of offspring. Thus, it is necessary to research the first offspring (F1) of the *Sorghum sudanense* irradiated by gamma rays offspring compared to *Sorghum sudanense* without radiation or with their parents as a basis for examining the next generation by paying attention to the right harvest *age* to achieve optimal productivity and quality.

Materials and Methods

This research was conducted from December 2019 to April 2020 at the pasture of the Faculty of Animal Science, UGM, Forage and Pasture Science Laboratory, and the Isotope and Radiation Technology Research and Development Center Laboratory, National Nuclear Energy Agency of Indonesia (BATAN), Pasar Jumat, South Jakarta. There were 5 stages of this research, namely land preparation, sorghum seeds planting, plant growth measurement, harvest production calculation, and laboratory analysis. Dry matter (DM) measurement was done by taking a sample of 1 gram. Then, it is put in an oven at 105ºC for 24 hours. The weight difference after and before the oven was calculated (AOAC, 2005). Organic matter (OM) measurement was carried out by putting the sample from the oven at 105ºC to the kiln. The weight difference before and after the kiln was measured (AOAC, 2005). Lignin measurement using *Chesson* (Datta, 1981) method by subtracting the sample residual weight after treatment with H_2SO_4 72% which was reduced by the sample residual ash. Then, divided by the initial dry weight of the sample and multiplied by 100%. Plant length was measured using a measuring tape. Plant length was measured starting from the soil surface to the tip of the longest leaf on each plant. Plant height was measured using a measuring tape. Plant height was measured starting from the soil surface to the highest point on each plant. The material used was *Sorghum sudanense* seeds from the first offspring (F1) were irradiated with gamma rays with a dose of 300 Gy and seeds (F1) without gamma irradiation, and sorghum plants were harvested at 50, 70, and 90 days old. The method used was gamma-ray irradiation using 300 Gy dose, planting, and harvesting. The data observed were plant growth, DM and OM production (ton/ha), and lignin content. Data were analyzed statistically with analysis of variance based on *split-plot design* 2x3. *The main plot* was sorghum varieties and *the subplot* was harvest *age*. Data with a significant difference as the treatment effect were further tested with *Duncan's Multiple Range Test* (DMRT) (Astuti, 1980).

Results and Discussion

Plant height

The plant height of *Sorghum sudanense* with gamma-ray irradiation and without gamma-ray irradiation at different harvest ages are presented in Table 1.

The plant height measurement results in the Table 1 showed a significant difference (P<0.05) between sorghum *varieties and harvest ages*. *Sorghum sudanense* plant height at the same age had a significant mean, where *Sorghum sudanense* with gamma-ray irradiation was higher than *Sorghum sudanense* without irradiation. The highest plant height mean was *Sorghum sudanense* with gamma-ray irradiation at 90 days old (266.92 cm). Sriagtula and Sowmen (2018) stated that the BMR Patir 3.2 mutant sorghum strain showed an average plant height at 92 days old was 117.92 cm. Furthermore, Astuti *et al*. (2019) stated that the height of the Numbu variety (non-mutant) at 70 days old was143.13 cm. *Sorghum sudanense* in this study, both mutant and conventional had a higher average plant height. Gamma-ray irradiation can assist in sorghum varieties assembly with high yield potential and well adapted to various agricultural agroecological conditions (Human, 2007). Paolella (1998) stated that gamma-ray irradiation affects the DNA or RNA base sequence. Changes

Table 1. Plant height (cm) of *Sorghum sudanense* with irradiation and no irradiation at different harvest ages

Sorghum Varieties	Harvest age (days)			Average
	50	70	90	
Irradiation	154.46 ± 3.78	209.09 ± 1.67	266.92 ± 10.17	210.16 ± 49.00^{x}
No irradiation	94.42 ± 4.48	156.48 ± 0.67	195.32 ± 4.86	148.90 ± 43.97^{y}
Average	124.69 ± 32.82^{k}	182.78 ± 28.84^{l}	231.12 ± 39.85^{m}	

^{k,l,m}*Different superscripts on the same line showed significant differences (P<0.05).*
^{x,y}*Different superscripts in the same column showed significant differences (P<0.05).*

in the bases sequence result in modifications in the amino acid sequence of a protein. Then, it performs in the behavior, morphology, and physiology changes of an individual. Plant morphology, including plant height, can change in a positive or negative direction, according to the nature of gamma-ray irradiation. Changes in a protein are closely related to the work of growth hormone, namely auxin. Auxin will stimulate proteins in the cell membrane to pump H+ ions to the cell wall. This H+ ion will activate the enzyme to break some of the hydrogen cross-links of the cellulose molecular chain. Plants then elongate due to water entering by osmosis. After this elongation, the cell continues to grow by re-synthesizing cell wall material and cytoplasm (Campbell *et al*., 2004). The gamma-ray irradiation will affect plant height and lead to positive results with the right dose. Therefore, *Sorghum sudanense* with gamma irradiation had a higher plant height than Sorghum without gamma irradiation. Plant height is a quantitative trait controlled by many genes. The plant height character or decrease in plant height is the most common indicator used to see the effects of mutagens both physically and chemically (Aisyah, 2006).

Plant length

The plant length of *Sorghum sudanense* with gamma-ray irradiation and without gamma-ray irradiation at different harvest ages are presented in Table 2.

The plant lengths presented in Table 2 showed significant yields (P<0.05) between sorghum varieties and harvest ages. *Sorghum sudanense* plant length at the same age had a significant mean, where *Sorghum sudanense* with gamma-ray irradiation was higher than *Sorghum sudanense* without irradiation. This proved that the right dose of gamma-ray irradiation will promote good plant genetic traits. There was an interaction (P<0.05) between varieties and harvest ages. The highest average was *Sorghum sudanense* with gamma irradiation at 90 days old (296.35 cm) and the lowest was *Sorghum sudanense* without gamma irradiation at 50 days old (127.30 cm). Plant length increased along with longer harvest age. This was in line with Crowder and Cheda (1982) statement where the longer the harvest age, the more opportunities for plants to grow and carry out photosynthesis. Hence, the accumulation of carbohydrates formed will be used for cell wall division and plant stem growth. Preussa and Britta (2003) stated plant stems can grow longer and also increase in diameter size. The existence of cells mutations caused by radiation can cause the irradiated cell division to be hindered when forming the division barrier process. Thus, in the anaphase, the cell division process cannot occur.

Dry matter and organic matter production

This study performed significant DM and OM production (P<0.05) between varieties and harvest ages. The dry matter production (ton/ha) in Table 3 shows a significantly different yield (P<0.05) between varieties and harvest ages. *Sorghum sudanense* with gamma-ray irradiation had a higher average DM production (4.33 tons/ha) than *Sorghum sudanense* without irradiation (1.75 tons/ha). Mugiono (2001) stated that the higher the dose, the more mutations occur and the more severe damage arises. The OM production in Table 4 also yielded significant results (P<0.05). The average OM production of *Sorghum sudanense* with irradiation (3.96 tons/ha) was higher than *Sorghum sudanense* without irradiation (1.63 tons/ha). The DM production (ton/ha) from sorghum with irradiation was higher than sorghum without irradiation. However, at 70 to 90 days old, there were production increases in sorghum without irradiation and production decreases in sorghum with irradiation. The same result happened as in the DM production (tons/ha).

The DM and OM production was influenced by the interaction between varieties and harvest ages (P<0.05). The highest DM production was *Sorghum sudanense* at 70 days

Table 2. Plant length (cm) of *Sorghum sudanense* with irradiation and no irradiation at different harvest ages

Sorghum Varieties	Harvest age (days)			Average
	50	70	90	
Irradiation	179.06 ± 5.34^{b}	238.55 ± 2.40^{d}	296.35 ± 5.00^{e}	237.99 ± 50.93^{x}
No irradiation	127.3 ± 0.15^{a}	175.89 ± 2.54^{b}	214.12 ± 14.59^{c}	172.45 ± 38.39^{y}
Average	153.20 ± 28.53^{k}	207.22 ± 34.38^{l}	255.24 ± 46.08^{m}	

^{a,b,c,d,e}Different superscripts in the same column and row showed significant differences (P<0.05).
^{k,l,m}Different superscripts on the same line showed significant differences (P<0.05).
^{x,y}Different superscripts in the same column showed significant differences (P<0.05).

Table 3. Dry matter production (ton/ha) of *Sorghum sudanense* with irradiation and no irradiation at different harvest ages

Sorghum Varieties	Harvest age (days)			Average
	50	70	90	
Irradiation	2.41 ± 0.15^{c}	5.64 ± 0.38^{f}	4.43 ± 0.39^{e}	4.33 ± 1.49^{x}
No irradiation	0.37 ± 0.04^{a}	1.12 ± 0.10^{b}	3.77 ± 0.60^{d}	1.75 ± 1.57^{y}
Average	1.39 ± 1.12^{k}	3.38 ± 2.48^{l}	4.35 ± 0.78^{m}	

$^{a, b, c, d, e, f}$ Different superscripts in the same column and row showed significant differences (P<0.05).
$^{k, l, m}$: Different superscripts on the same line showed significant differences (P<0.05).
$^{x, y}$: Different superscripts in the same column showed significant differences (P<0.05).

Table 4. Dry matter production (ton/ha) of *Sorghum sudanense* with irradiation and no irradiation at different harvest ages

Sorghum Varieties	Harvest age (days)			Average
	50	70	90	
Irradiation	2.16 ± 0.12[c]	5.18 ± 0.35[f]	4.54 ± 0.38[e]	3.96 ± 1.40[x]
No irradiation	0.33 ± 0.30[a]	1.04 ± 0.96[b]	3.52 ± 0.56[d]	1.63 ± 1.48[y]
Average	1.24 ± 1.00[k]	3.11 ± 2.28[l]	4.03 ± 0.70[m]	

[a,b,c,d,e,f] Different superscripts in the same column and row showed significant differences (P<0.05).
[k,l,m] Different superscripts on the same line showed significant differences (P<0.05).
[x,y] Different superscripts in the same column showed significant differences (P<0.05).

old (5.64 ton/ha), while the lowest yield was *Sorghum sudanense* without gamma irradiation 50 days old (0.37 ton/ha). The OM production showed the best interaction of *Sorghum sudanense* with gamma irradiation at 70 days old (5.18 ton/ha). Jung (2012) stated that plant maturity level affects the stems mass accumulation and exceeds the leaves mass accumulation. Based on Miron *et al.* (2006) research, DM production decreases with increasing plant age, in contrast to Atis *et al*. (2012) opinion, where increasing plant maturity will improve fresh production and DM production of sorghum plants This is due to longer the harvest age, the plant performs extended photosynthesis. The photosynthetic accumulation results are found in plant tissues. Gardner *et al.* (2008) stated that the longer the assimilation occurs, the higher the dry weight of the plant. Sitompul and Guritno (1995) stated that greater production of photosynthetic leads to the perfect formation of plant body parts such as stems, leaves, and roots. This can increase DM production which will be followed by OM production improvement. *The longer harvest age of Sorghum sudanense* without irradiation, especially at 70 to 90 days old, performed significant increases. Another factor can occur because each plant has a different harvest age to reach the peak of harvest production and decrease as the plant becomes older. Harjadi (2002) stated that production will be achieved at a high population, but will eventually decrease with increasing plant population due to competition for light and other growth factors.

Lignin content

Lignin content *Sorghum sudanense* with gamma-ray irradiation and without gamma-ray irradiation at different harvest ages are presented in Table 5.

The results obtained based on Table 5 showed that the lignin content had significant differences (P<0.05) from the varieties and harvest ages. The average lignin content of *Sorghum sudanense* with irradiation (3.99%) was lower than *Sorghum sudanense* without irradiation (7.86%). This indicated that sorghum with gamma-ray irradiation can reduce the lignin content. This decrease was due to genetic changes in plants from gamma-ray irradiation which can affect enzyme synthesis in plants. The low lignin content in the BMR strain was related to enzyme activity expression for lignin biosynthesis. The mutations result of the BMR mutant line decreased the *cinnamyl alcohol dehydrogenase* (CAD) and *caffeic acid O-methyltransferase* (COMT) enzyme activity (Li *et al*. 2015), thereby reducing the lignin content and increasing the glucose content (Scully *et al*. 2016). Halpin *et al*. (1998) stated *that brown-midrib* (BM1) in maize showed considerably reduced CAD activity in lignified tissue. It resulted in modified lignin production, both total lignin content and altered polymer structure due to mutations. Lehninger (1982) stated that mutations can cause several enzymes cannot be synthesized in an active form, resulting in the accumulation and excretion of substrates by damaged enzymes. Genetic abnormalities in these metabolic pathways can change the structure of specific genes in DNA.

The lignin content was affected (P<0.05) by the interaction between varieties and harvest ages. The lowest lignin was in *Sorghum sudanense* with gamma irradiation (3.52%), while the highest lignin was in *Sorghum sudanense* without gamma irradiation (10.00%). The lignin content increases at each harvest age due to the plant maturity level. As the plant matures, the lignin content will increase to support the growing plant. The stem contains a higher proportion of thick-walled tissue and less photosynthetic tissue than leaves, so the stem has a higher cell wall concentration than leaves (Wilson and Kennedy, 1996).

Table 5. Lignin content (%DM) of *Sorghum sudanense* with irradiation and no irradiation at different harvest ages

Sorghum Varieties	Harvest age (days)			Average
	50	70	90	
Irradiation	3.52 ± 0.86[a]	3.63 ± 0.24[a]	4.82 ± 0.13[b]	3.99 ± 0.64[x]
No irradiation	4.70 ± 0.52[b]	8.87 ± 0.27[c]	10.00 ± 0.51[d]	7.86 ± 2.45[y]
Average	4.11 ± 0.72[k]	6.25 ± 2.88[l]	7.41 ± 2.85[m]	

[a,b,c,d] Different superscripts in the same column and row showed significant differences (P<0.05).
[k,l,m] Different superscripts on the same line showed significant differences (P<0.05).
[x,y] Different superscripts in the same column showed significant differences (P<0.05).

Conclusions

In conclusion, *Sorghum sudanense* with gamma-ray irradiation had different properties with *Sorghum sudanense* without gamma-ray irradiation. Plant growth of *Sorghum sudanense* with gamma irradiation had a higher average than *Sorghum sudanense* without gamma irradiation. The highest production of *Sorghum sudanense* with gamma radiation was at 70 days old with a lignin content of 3.63%.

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