p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Strengthening for Research and Development No: 30/E/KPT/2018

Tropical Animal Science Journal, September 2022, 45(3):327-336
DOI: https://doi.org/10.5398/tasj.2022.45.3.327
Available online at http://journal.ipb.ac.id/index.php/tasj

Morphological Characteristics and Nutritional Quality of Mutant Benggala Grass (*Panicum maximum* cv Purple Guinea) Generation M1V3

A. Fanindi[a,*], S. H. Sutjahjo[b], S. I. Aisyah[b], & N. D. Purwanti[a]
[a]Indonesian Research Institute of Animal Production
Jalan Banjarwaru, PO Box 221, Ciawi Bogor 16002, West Java, Indonesia
[b]Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University
Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia
*Corresponding author: afanindi@gmail.com
(Received 24-05-2021; Revised 07-12-2021; Accepted 20-12-2021)

ABSTRACT

This study aims to observe morphological characters and genetic parameters of Benggala grass and its nutritional quality. Research on morphological characters was conducted at the Regional Technical Executive Unit (UPTD) of Tenjo dry land, using a randomized block design with 5 replications. The parents of the M1V3 mutant were from Benggala grass cv Purple guinea from the germplasm collection of the Indonesian Research Institute of Animal Production (Balitnak). The M1V3 plants were derived from 2400 M1V1 plants sorted to 250 M1V2 plants, and finally to 29 M1V3 plants. There were 29 plants planted on the experimental field using pols and control plants. Morphological observations and forage harvests were conducted at harvest/cutting ages 2 and 3 months after planting. Each harvest age was analyzed respectively. Forage quality observations were carried out at the RIAP Laboratory. The results showed that at the ages of 2 and 3 months, almost all characters were significantly different ($p<0.05$) in each genotype and several mutants of Benggala grass were higher than controls. Broad categorical genetic parameters were found in the characters of fresh weight, fresh weight of leaves, and fresh weight of tillers. Nutritional quality shows that irradiated plants have good nutritional quality because the value of crude protein and digestibility increases, while the value of crude fiber decreases compared to the control plant. In conclusion, the characterization of the M1V3 generation showed high-yielding mutants that were higher than the control and this M1V3 generation could be used as candidates for high-yielding varieties of Benggala grass.

Keywords: Benggala grass; genetic; nutrition value; morphology

INTRODUCTION

Benggala grass is a common forage among Indonesian farmers (Fanindi *et al.,* 2019). Mutation breeding to increase the production of Benggala grass has not been widely practiced in Indonesia, whereas mutation breeding has been used to create plant varieties with desirable traits such as yield, pest resistance, and tolerance to abiotic stresses. Increasing the production and nutritional value of forage becomes important, because the number of livestock increases. In addition, farmers in developing countries, such as Indonesia, have a narrow land area that requires farmers to plant grasses with high productivity and nutritional value.

Mutation breeding in Benggala grass using gamma radiation is expected to produce varieties with higher productivity than their parents. The M1V3 mutant of Benggala grass in this study resulted from the selection of the M1V1 and M1V2 generations, which were previously irradiated using gamma rays at a dose of 200-350 Gy. Research on the productivity and quality of Benggala grass was carried out on vegetative generation 3 (MV3) mutants because plants in the MV3 generation vegetatively grown are usually genetically stable and will not change (Aisyah, 2009). Observation of genetic parameters was conducted to find genotypes according to the goals of the breeders. This parameter is expected to be able to select the genotype at each stage of selection (Zen, 2012). Furthermore, principal component analysis (PCA) derived valuable information from the sample and represented the largest variance (Song & Li, 2021). Galo *et al.* (2015) used PCA on biological characters to differentiate low- and high-quality forages. In addition, PCA could be used to determine the chemical relationship (protein, lignin), carbohydrates, and digestibility of forage tested. Principal component analysis (PCA) was also used to analyze characters related to oil production in vetiver grass genotypes (Lal *et al.,* 2018).

The nutritional quality of forage is an important factor besides production because the quality of forage directly impacts livestock productivity. High quality and production of forage are factors for the success of the livestock industry. Besides, the nutritional value of livestock is the basis for preparing livestock rations. However, forage production and quality vary greatly between species and are affected by harvest season and age (Zhang *et al.*, 2018). As a result, in this MV3 generation, it is important to study the productivity of Benggala grass forage at various harvest ages to determine its productivity. This study aims to determine the morphological characteristics, genetic variability, and forage quality of the third-generation mutant (M1V3) of Benggala grass. The results of this study are expected to obtain superior Benggala grass with high production and quality so that it can provide forage needs and increase livestock productivity.

MATERIALS AND METHODS

Identification of Morphological Characteristics of M1V3

The research was conducted at UPTD dry land, Kecamatan Tenjo, Bogor Regency for 4 months. Genetic material (seeds) was taken from the germplasm of the experimental garden of the Research Institute of Animal Production (RIAP). The plant material used was selected individually in the M1V2 generation. The M1V2 plants were derived from the M1V1 selection based on a minimum 20% higher production compared to the original plants. The selection method used is clone selection. The number of selected M1V1 plants was 2400, then 250 plants were selected in the M1V2 generation, and 29 plants were selected to be planted in the M1V3 generation.

The M1V3 plants were then observed for morphological and nutritional values and production to produce superior clones of Benggala grass. Seedlings of M1V3 were sown in polybags and transferred to the planting plot after one month. This study used a completely randomized block design (CRBD) with five replications, while grouping was based on land conditions or slope of the land. The treatments consisted of 29 different mutant plants and a control variety. Mutant plants and control varieties were planted on the plots size of 1.5 m x 15 m. Fertilization was given with a dose of 100 kg/ha for Urea, 100 kg/ha for TSP, 100 kg/ha for KCL, and 10 tons/ha for manure.

Harvesting/cuttings of forage were done 2 and 3 months after planting. The morphological characteristics observed were plant height, stem height, leaf length, leaf width, internode length, stem diameter, fresh weight of forage, dry weight of forage, root weight, and root length. The nutritional contents of the mutant plants were also observed.

Estimation of genetic parameters was conducted based on Singh and Chaudhary's formula (Singh & Caudhary, 1977) with the following formulas:

Genotype variance (σ^2g)= M2-M1/r;
Phenotype variance (σ^2p)= σ^2g + M1; and
Heritability (h^2bs) = σ^2g/σ^2p.

The coefficient of the genetic variable was also calculated. The genetic variable of a character was determined based on the genetic variance and the standard deviation of genetic variance, if σ^2g> 2 $\sigma\sigma^2$g: the genetic diversity is wide, while if σ^2g <2 $\sigma\sigma^2$ g: the genetic variable is narrow (Pinaria *et al.*, 1996). The standard deviation of genetic variance is based on the following formula:

$$\sigma_{\sigma}^2\ g = \sqrt{\frac{2}{(r)^2}\left[\frac{{M_2}^2}{dbg+2} + \frac{{M_1}^2}{dbe+2}\right]}$$

The similarity and variance of accessions were analyzed using hierarchical cluster analysis with cluster heatmaps and principal component analysis using R 3.4.

Analysis of Forage Nutritional Quality

The forage of Benggala grass harvested at the age of two months was used to measure the nutritional content. The plants analyzed were selected plants that had high production at each harvest age. The test was conducted at the Laboratory of the Research Institute of Animal Production. The nutritional contents observed were crude protein, energy, crude fiber, dry matter and organic matter digestibility. The method used to analyze crude protein was the Kjedhal method (Thiex *et al.*, 2002), crude fiber was measured using the Van Soest method (Van Soest *et al.*, 1991), the digestibility of dry matter and organic matter was measured in vitro using the Tilley & Terry method (Tilley & Terry, 1963).

RESULTS

Identification of Morphological Characteristics

The results of the study showed that all characters, such as plant height, stem height, stem diameter, number of tillers, fresh weight, fresh leaf weight, and leaf weight percentage, were significantly ($p<0.05$) affected by the genotype (mutant number) (Tables 1 and 3). Therefore, genotypes with high fresh and dry weights could be developed into new superior forage varieties. Mutant plants obtained had a higher forage weight and the number of tillers compared to the control ($p<0.05$) (Table 2). The number of mutants that had the number of tillers, fresh weight, and percentage of leaf weight higher than the control at 2 months of cutting age were mutant numbers 12, 5, 7, and 22, mutant numbers 11 and 7, and mutant numbers 4, 12, and 22, respectively. Meanwhile, at 3 months of cutting age, the mutants with the highest number of tillers, fresh leaf weight, and dry leaf weights were mutant numbers 5 and 12, mutant numbers 6, 12, and 22, and mutant numbers 22 and 12, respectively. These mutants have the potential to be used as new superior varieties of Benggala grass that have high productivity (Table 4).

The genetic variances of plants at the cutting ages of 2 and 3 months were presented in Table 5. High heritability at 2 months of harvesting was found in the number of tillers, length of flag leaves, total fresh weight, and fresh weight of leaves. Meanwhile, at 3 months of harvesting, the high heritability was found in leaf width, fresh leaf weight, percentage of leaf fresh weight, and leaf dry weight. Low heritability at 2 months of harvesting was recorded in dry weight, root length, and dry leaf weight; whereas at 3 months of cutting age, the length of the internodes and roots had low heritability.

Table 1. Averages plant height, stem height, stem diameter, internode length, leaf length, leaf width, and lengths of flag leaves of Benggala grass at 2 months of cutting age

Genotype	Variables						
	Plant height (cm)	Stem height (cm)	Stem diameter (mm)	Internode length (cm)	Leaf length (cm)	Leaf width (cm)	Lengths of flag leaves (cm)
1	203.6±3.$^{1a-c}$	151.8±12.6^{ab}	5.73±1.1^{b-e}	29.2±6.7^{a-e}	44.6±5.5^{a-d}	2.3±0.3^{a-d}	13.2±3.1^{a-f}
2	204.4±5.$^{3a-c}$	144.2±10.8 $^{a-c}$	5.76±1.2^{b-e}	32.4±4.4^{a-d}	47.8±3.5^{a-c}	2.7±0.3^{ab}	16.6±4.2^{a}
3	193.6±12.5^{a-c}	140.0±14.1 $^{a-c}$	5.42±1.1^{c-e}	29.6±6.3^{a-e}	47.4±6.5^{a-c}	2.4±0.2^{a-d}	13.1±4.2^{a-f}
4	193.6±16.1^{a-c}	125.6±13.9^{cd}	5.70±0.9^{b-e}	30.8±2.9^{a-e}	44.2±2.9^{a-d}	2.4±0.4^{a-d}	12.4±4.5^{a-g}
5	166.8±11.9^{d}	114.2±12.1^{d}	5.06±0.9^{e}	23.2±4.7^{f}	40.6±21^{cd}	2.3±0.3^{a-d}	7.6±2.1^{fg}
6	193.2±13.3^{a-c}	135.4±19.8^{a-d}	5.81±1.0^{b-e}	30.2±5.1^{a-e}	45.0±4.8^{a-d}	2.2±0.2^{a-d}	8.2±2.5^{e-g}
7	200.6±8.9^{a-c}	148.4±17.5^{a-c}	5.66±0.7 $^{b-e}$	29.8±0.8^{a-e}	48.0±5.1^{a-c}	2.2± 0.5^{cd}	13.6±3.3^{a-e}
8	196.0±5.1^{a-c}	139.4±11.7^{a-c}	5.30±0.5^{de}	26.0±2.7^{d-f}	50.6±8.5^{a-c}	2.6±0.4^{a-c}	12.4±3.3^{a-g}
9	201.8±11.7^{a-c}	149.6±11.6^{a-c}	5.26±0.5^{de}	30.0±4.0^{a-e}	45.4±8.9^{a-d}	2.4±0.3^{a-d}	13.4±3.1^{a-f}
10	205.4±8.9^{a-c}	143.2±13.4^{a-c}	6.01±0.9^{a-e}	29.6±2.7^{a-e}	52.6±7.4^{ab}	2.6±0.4^{a-c}	11.4±2.1^{a-g}
11	210.2±13.4^{ab}	141.0±16.9^{a-c}	5.78±0.8 $^{b-e}$	27.2±3.0^{c-f}	41.6±7.6^{b-d}	2.5±0.4^{a-c}	11.6±3.5^{a-g}
12	193.6±14.3^{a-c}	137.4±15.1^{a-d}	5.43±1.1^{c-e}	24.8±5.6^{ef}	36.0±6.2^{d}	2.6±0.4^{a-c}	7.2±2.0^{g}
13	200.8±10.3^{a-c}	135.4±5.3^{a-d}	5.93±0.7^{a-e}	31.2±4.9^{a-e}	47.0±7.6^{a-c}	2.4±0.2^{a-d}	17.0±3.3^{a}
14	189.4±16.3^{a-d}	137.6±15.1^{a-d}	5.58±0.5^{b-e}	29.2±2.2^{a-e}	48.0±4.2^{a-c}	2.3±0.2^{a-d}	13.6±2.0^{a-e}
15	190.6±14.2^{a-d}	136.8±12.9^{a-d}	5.01±0.8^{e}	31.0±6.7^{a-e}	49.0±7.1^{a-c}	2.5±0.4^{a-d}	9.8±2.3^{b-g}
16	209.0±10.7^{a-c}	155.0±13.2^{a}	6.34±0.9^{a-d}	29.8±4.2^{a-e}	44.6±5.3^{a-d}	2.5±0.3^{a-d}	8.6±2.3^{d-g}
17	213.6±14.8^{a}	140.6±7.6^{a-c}	5.49±1.1^{c-e}	32.8±5.8^{a-c}	47.0±9.3^{a-c}	2.4±0.4^{a-d}	15.4±4.1^{ab}
18	203.2±13.3^{a-c}	134.6±8.7^{a-d}	5.79±1.8^{b-e}	30.2±2.3^{a-e}	52.2±5.6^{ab}	2.5±0.3^{a-d}	16.8±3.9^{a}
19	202.2±11.8^{a-c}	142.2±4.1^{a-c}	5.48±1.2^{c-e}	33.8±4.8^{ab}	43.0±7.9^{b-d}	2.5±0.4^{a-d}	12.2±2.8^{a-g}
20	194.8±7.12^{a-c}	137.6±15.2^{a-d}	6.60±1.3^{a-c}	29.8±3.1^{a-e}	48.2±2.4^{a-c}	2.5±0.4^{a-c}	11.6±3.0^{a-g}
21	201.2±6.3^{a-c}	138.2±15.5^{a-d}	6.07±0.4^{a-e}	29.8±4.8^{a-e}	47.2±7.5^{a-c}	2.4±0.3^{a-d}	11.5±3.0^{a-g}
22	197.0±6.0^{a-c}	130.8±7.4^{a-d}	6.06±1.1^{a-e}	31.0±1.2^{a-e}	48.0±7.2^{a-c}	2.00±0.3^{d}	8.8±1.6^{c-g}
23	185.6±14.1^{b-d}	149.2±11.0^{a-c}	6.72±0.9^{ab}	34.4±2.9^{a}	48.2±9.6^{a-c}	2.6±0.1^{a-c}	14.4±3.5^{a-d}
24	206.6±16.7^{a-c}	148.6±14.0^{a-c}	5.57±0.8 $^{b-e}$	30.6±3.2^{a-e}	48.6±5.6^{a-c}	2.7±0.2^{a}	13.6±2.0^{a-e}
25	195.2±6.7^{a-c}	129.0±15.4^{b-d}	5.43±0.8^{c-e}	31.8±6.2^{a-d}	44.0±6.8^{a-d}	2.2±0.2^{b-d}	13.8±3.4^{a-e}
26	187.4±11.6^{a-d}	138.6±11.8^{a-c}	5.75±1.1^{b-e}	27.8±4.1^{b-f}	46.4±5.9^{a-d}	2.5±0.3^{a-c}	10.7±3.3^{b-g}
27	192.2±16.2^{a-c}	124.8±5.8^{cd}	6.06±1.1^{a-e}	29.6±5.0^{a-e}	49.0±2.9^{a-c}	2.6±0.2^{a-c}	11.6±3.1^{a-g}
28	183.4±16.6^{cd}	133.6±11.8^{a-d}	6.19±1.2^{a-e}	29.2±3.7^{a-e}	54.6±9.6^{a}	2.5±0.1^{a-c}	15.6±3.8^{ab}
29	193.6±10.4^{a-c}	136.4±13.5^{a-d}	7.05±0.7^{a}	29.4±7.7^{a-e}	46.2±10.8^{a-d}	2.6±0.2^{a-c}	14.6±3.1^{a-c}
C	200.6±12.4^{a-c}	142.0±20.4^{a-c}	5.47±0.7^{c-e}	31.2±3.3^{a-e}	45.4±7.7^{a-d}	2.3±0.2^{a-d}	13.6±2.5^{a-e}

Note: Means in the same columns with different superscripts differ significantly ($p<0.05$). a-c= abc; a-d= abcd; a-e= abcde; a-f= abcdef; a-g= abcdefg; b-d= bcd; b-e= bcde; b-f= bcdef; b-g= bcdefg; c-e= cde; c-f= cdef; c-g= cdefg; d-f= def; d-g= defg.

The broad genetic variances in the mutant at 2 months of cutting age were found in the number of tillers, length of flag leaves, fresh weight, leaf weight, and percentage of fresh weight. The values of broad genetic variances in the mutant at the 3 months of cutting age were found in the number of tillers, dry weight, fresh leaf weight, and dry weight.

The analysis results showed that the principal component analysis (PCA) reduced the observed characters to 4 main components, which could determine the diversity of 30 Benggala grass numbers by 70.8% (Figure 1). The number of tillers, fresh forage weight, fresh leaf weight, dry leaf weight, and root weight were the characteristics that influenced the first principal component (PC-1). Characteristics such as leaf length, flag leaf length, and flag leaf width contribute to the diversity in principal component 2 (PC-2), while plant height and stem height contribute to the diversity in principal component 3 (PC-3), and the diameter of the stem contributes to the diversity in PC-4.

The results of the principal component analysis also showed that mutants had a positive correlation with PC-1 and PC-2, including the mutant numbers 20, 23.8, 29, and 25 (Table 6). This mutant was correlated with the characteristics in this quadrant of leaf length, internode length, leaf width, plant height, stem height, as well as length and width of high flag leaves (Figure 2). While the mutants that were positively correlated with PC-2 (quadrant II) were plant numbers 22, 6, 1, 11, and 21, and the highly correlated characteristics in this quadrant were fresh green weight, fresh leaf weight, dry leaf weight, root weight, and root length. Mutants in quadrant III were correlated with tiller characteristics and the high percentage of fresh leaf weight, namely plant numbers 14, 4, 5, 3 and 12, 18, 24, 27.

The results of the principal component analysis also showed that the selection characteristics that had a positive correlation with fresh forage weight were fresh leaf weight, dry leaf weight, root weight, and root length. This characteristic can be used as an indirect character to select high-producing Benggala grass.

Table 2. Averages width of flag leaves, number of tillers, fresh weight, dry weight, fresh leaf weight, dry leaf weight, percentage of weight leaf, and root length of Benggala grass at 2 months of cutting age

Genotype	Variables							
	Width of flag leaves (cm)	Number of tillers	Fresh weight (g/plant)	Dry weight (g/plant)	Fresh leaf weight (g/plant)	Dry leaf weight (g/plant)	Percentage of weight leaf (%)	Root length (cm)
1	1.3±0.3^{a-c}	38.6±8.7^{a-d}	1072.0±103.5^{a-c}	209.0±28.8^{ab}	360.4±31.4^{a-e}	69.6±12.2^{a-c}	31.9±5.6^{b-d}	35.8^{a-c}
2	1.6±0.3^{a}	31.8±3.8^{c-f}	1018.0±93.9^{a-e}	201.4±25.4^{a-c}	272.4±52.6^{a-e}	54.0±9.9^{a-d}	24.4±5.5^{d}	34.0^{a-d}
3	1.3±0.4^{a-c}	28.4±6.5^{d-f}	884.0±67.3^{b-f}	165.0±51.3^{a-c}	283.4±56.9^{a-e}	52.8±7.6^{a-d}	32.2±9.5^{b-d}	27.2^{d}
4	1.4±0.4^{a-c}	25.2±4.6^{ef}	676.0±122.1^{g-f}	131.0±47.1^{bc}	324.0±59.5^{a-e}	64.0±12.3^{a-d}	51.5±8.2^{a}	32.4^{a-d}
5	0.8±0.2^{c}	44.8±9.6^{ab}	810.0±129.8^{c-g}	139.8±36.9^{bc}	316.8±38.9^{a-e}	56.4±14.9^{a-d}	40.1±8.4^{a-d}	32.0^{a-d}
6	0.8±0.1^{c}	30.8±5.0^{c-f}	868.0±120.7^{b-f}	157.6±59.8^{a-c}	327.0±34.6^{a-e}	61.2±3.8^{a-d}	41.1±8.4^{a-d}	35.0^{a-d}
7	1.4±0.3^{ab}	45.4±5.1^{a}	1138.0±95.6^{ab}	251.4±53.0^{a}	343.6±54.5^{a-e}	63.4±11.5^{a-d}	28.8±7.7^{cd}	30.4^{a-d}
8	1.1±0.1^{a-c}	32.2±4.2^{c-f}	950.0±106.3^{a-f}	159.4±15.9^{a-c}	379.6±27.6^{a-d}	68.8±12.9^{a-d}	42.5±10.1^{a-d}	38.2^{a}
9	1.3±0.4^{a-c}	26.6±5.5^{d-f}	826.0±96.3^{c-f}	174.6±24.3^{a-c}	324.2±48.7^{a-e}	57.4±14.5^{a-d}	28.7±8.4^{cd}	28.4^{b-d}
10	1.0±0.3^{a-c}	27.8±6.2^{d-f}	900.0±147.4^{b-f}	166.2±56.1^{a-c}	306.2±39.6^{a-e}	51.4±10.0^{a-d}	30.5±7.7^{b-d}	33.2^{a-d}
11	1.1±0.3^{a-c}	33.6±5.4^{b-f}	1202.0±73.96^{a}	199.2±64.4^{a-c}	401.4±46.0^{a-c}	70.8±12.7^{a-c}	28.2±4.2^{cd}	32.0^{a-d}
12	0.8±0.2^{c}	44.4±4.8^{ab}	938.0±121.3^{a-f}	171.6±60.6^{a-c}	420.2±43.3^{ab}	77.0±8.4^{ab}	47.5±8.9^{a-c}	28.6^{b-d}
13	1.3±0.4^{a-c}	27.6±6.3^{d-f}	998.0±130.6^{a-e}	155.8±53.5^{bc}	363.8±50.7^{a-d}	58.6±7.2^{a-d}	26.3±5.7^{d}	32.6^{a-d}
14	1.3±0.3^{a-c}	35.2±6.6^{a-e}	852.0±97.0^{b-f}	147.0±44.2^{bc}	278.0±37.8^{a-e}	56.2±4.2^{a-d}	34.8±6.3^{a-d}	31.6^{a-d}
15	0.9±0.2^{bc}	27.8±7.7^{d-f}	964.0±123.0^{a-f}	140.2±31.0^{bc}	293.6±42.9^{a-e}	52.4±2.9^{a-d}	32.4±7.2^{b-d}	32.4^{a-d}
16	1.0±0.3^{bc}	26.2±7.9^{d-f}	798.0±144.1^{a-c}	158.6±54.8^{a-c}	292.8±32.8^{a-e}	56.6±9.6^{a-d}	36.8±6.0^{a-d}	29.4^{a-d}
17	1.5±0.4^{ab}	27.6±5.2^{d-f}	956.0±115.8^{a-f}	171.8±64.9^{a-c}	291.8±18.9^{a-e}	54.4±12.2^{a-d}	27.3±4.8^{d}	31.4^{a-d}
18	1.3±0.3^{a-c}	28.2±7.5^{d-f}	936.0±62.7^{a-f}	183.2±53.3^{a-c}	251.8±49.0^{a-e}	49.8±7.2^{a-d}	30.9±2.6^{b-d}	30.4^{a-d}
19	1.0±0.2^{a-c}	25.0±8.1^{ef}	548.0±114.3^{g}	106.4±36.1^{c}	164.8±53.5^{e}	32.8±11.9^{d}	29.9±6.5^{b-d}	30.0^{a-d}
20	1.2±0.2^{a-c}	27.8±2.6^{d-f}	924.0±72.3^{a-f}	169.8±21.6^{a-c}	267.4±50.9^{a-e}	46.0±9.1^{a-d}	27.1±5.3^{d}	37.2^{ab}
21	1.0±0.1^{bc}	29.8±4.6^{c-f}	866.0±59.4^{b-f}	155.8±44.7^{bc}	310.2±25.3^{a-e}	57.6±14.9^{a-d}	29.8±4.0^{b-d}	32.6^{a-d}
22	0.9±0.1^{bc}	40.6±7.7^{a-c}	926.0±85.0^{a-f}	187.2±60.1^{a-c}	438.6±24.0^{a}	82.0±13.1^{a}	49.2±9.5^{ab}	28.6^{b-d}
23	1.3±0.3^{a-c}	29.4±6.4^{c-f}	1038.0±55.9^{a-d}	173.2±59.9^{a-c}	287.2±43.4^{a-e}	54.6±16.7^{a-d}	27.2±9.1^{d}	34.4^{a-d}
24	1.2±0.4^{a-c}	22.6±3.4^{f}	800.0±84.2^{c-g}	134.2±33.4^{bc}	204.4±26.2^{de}	39.8±8.3^{cd}	23.9±6.8^{d}	33.0^{a-d}
25	1.4±0.4^{ab}	27.8±9.0^{d-f}	726.0±120.5^{e-g}	123.2±43.9^{bc}	213.0±44.8^{b-e}	39.4±7.4^{cd}	27.8±5.2^{d}	31.8^{a-d}
26	1.1±0.4^{a-c}	27.8±5.7^{d-f}	756.0±94.3^{d-g}	136.0±37.9^{bc}	238.6±41.8^{b-e}	47.8±8.9^{a-d}	28.3±6.4^{cd}	28.0^{b-d}
27	1.3±0.5^{a-c}	24.6±3.3^{ef}	978.0±102.6^{a-e}	173.4±39.6^{a-c}	313.4±45.7^{a-e}	58.0±13.1^{a-d}	23.3±5.4^{d}	25.8^{d}
28	1.4±0.4^{a-c}	32.2±8.1^{c-f}	1084.0±70.9^{a-c}	187.4±22.2^{a-c}	339.8±31.2^{a-e}	64.2±11.7^{a-d}	29.3±c6.8^{d}	29.4^{a-d}
29	1.4±0.4^{a-c}	28.0±7.3^{d-f}	792.0±19.2^{c-g}	142.6±38.4^{bc}	257.8±46.1^{a-e}	42.0±15.0^{b-d}	32.0±6.8^{b-d}	31.0^{a-d}
C	1.2±0.3^{a-c}	28.4±6.6^{d-f}	882.0±58.7^{b-f}	170.4±28.8^{a-c}	209.4±54.5^{c-e}	46.4±15.9^{a-d}	25.7±4.6^{d}	30.8^{a-d}

Note: Means in the same columns with different superscripts differ significantly ($p<0.05$). a-c= abc; a-d= abcd; a-e= abcde; a-f= abcdef; a-g= abcdefg; b-d= bcd; b-e= bcde; b-f= bcdef; b-g= bcdefg; c-e= cde; c-f= cdef; c-g= cdefg; d-f= def; d-g= defg.

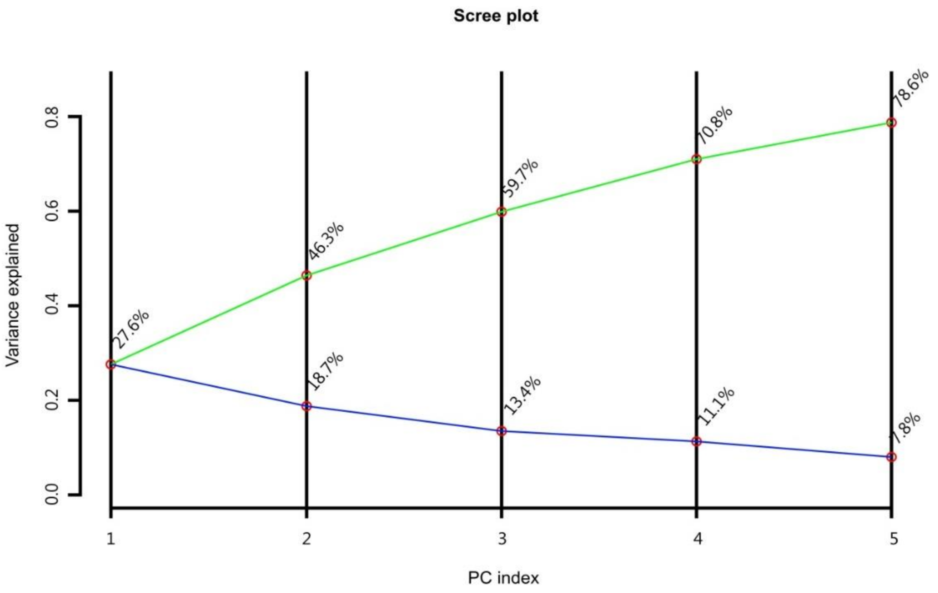

Figure 1. Percentage of diversity based on morphological characters of 30 Benggala grass mutants

Table 3. Averages plant height, stem height, stem diameter, internode length, leaf length, leaf width, length of the leaves flag, and width of the leaves flag of Benggala grass at 3 months of cutting age

Genotype	Variables							
	Plant height (cm)	Stem height (cm)	Stem diameter (mm)	Internode length (cm)	Leaf length (cm)	Leaf width (cm)	Length of the leaves flag (cm)	Width of the leaves flag (cm)
1	200.4±10.7^{ab}	131.4±9.4^{b}	5.5±0.3^{a-d}	31.4±5.9^{ab}	37.2±2.6^{a-d}	2.4±0.1^{de}	16.2±3.1^{a-e}	1.7±0.2^{ab}
2	213.0±4.6^{ab}	150.4±8.7^{ab}	5.6±0.5^{a-d}	35.4±4.3^{a}	35.0±4.9^{a-d}	2.6±0.5^{a-d}	15.0±3.7^{a-f}	1.6±0.2^{ab}
3	193.4±13.8^{b}	138.4±7.9^{ab}	5.9±0.5^{a-d}	27.2±4.3^{ab}	29.0±1.7^{cd}	2.1±0.3^{e}	12.0±2.9^{c-f}	1.1±0.1^{ab}
4	203.8±17.8^{ab}	142.6±7.4^{ab}	5.2±0.5^{a-d}	28.4±3.9^{ab}	35.4±4.6^{a-d}	2.4±0.4^{ced}	14.1±1.9^{a-f}	1.3±0.1^{ab}
5	189.8±19.6^{b}	134.4±8.2^{ab}	5.1±0.4^{a-d}	22.8±4.6^{b}	33.2±2.7^{a-d}	2.7±0.4^{a-d}	13.2±4.2^{b-f}	1.5±0.2^{ab}
6	205.4±11.9^{ab}	147.8±10.9^{ab}	5.5±0.5^{a-d}	27.0±7.5^{ab}	35.0±5.9^{a-d}	2.7±0.2^{a-d}	15.4±4.4^{a-f}	1.7±0.2^{ab}
7	200.2±1.7^{ab}	162.0±9.5^{a}	5.6±0.4^{a-d}	29.6±8.3^{ab}	40.6±4.2^{a-d}	2.8±0.5^{a-d}	13.6±3.6^{a-f}	1.5±0.3^{ab}
8	192.8±9.4^{b}	148.4±8.0^{ab}	4.8±0.8^{cd}	30.8±7.5^{ab}	43.0±3.3^{ab}	2.7±0.4^{a-d}	19.2±3.8^{ab}	1.9±0.2^{a}
9	202.2±8.4^{ab}	147.6±12.6^{ab}	5.6±0.5^{a-d}	31.8±2.5^{ab}	34.6±4.1^{a-d}	2.6±0.3^{a-d}	12.6±2.1^{b-f}	1.3±0.3^{ab}
10	204.4±11.2^{ab}	130.4±9.0^{b}	6.2±0.4^{a}	31.8±2.4^{ab}	44.4±6.2^{ab}	2.9±0.3^{a-d}	16.0±3.7^{a-f}	1.6±0.2^{ab}
11	226.2±17.3^{a}	164.0±5.7^{a}	5.7±0.7^{a-d}	25.0±4.7^{ab}	38.4±5.9^{a-d}	2.8±0.2^{a-d}	10.8±3.8^{d-f}	1.5±0.3^{ab}
12	200.4±14.2^{ab}	135.6±8.9^{ab}	4.8±0.5^{cd}	26.6±7.9^{ab}	27.4±5.7^{d}	2.5±0.5^{bced}	9.6±2.9^{ef}	0.9±0.2^{b}
13	188.2±6.5^{b}	134.6±10.1^{ab}	4.9±0.6^{b-d}	31.8±5.1^{ab}	37.0±5.3^{a-d}	2.7±0.2^{a-d}	14.2±3.9^{a-f}	1.5±0.3^{ab}
14	200.8±13.3^{ab}	135.8±13.9^{ab}	5.6±0.2^{a-d}	25.2±5.9^{ab}	32.0±4.8^{b-d}	2.5±0.3^{b-d}	12.4±3.8^{c-f}	1.3±0.2^{ab}
15	207.4±17.4^{ab}	162.6±9.2^{a}	5.5±0.6^{a-d}	30.8±6.4^{ab}	33.8±4.2^{a-d}	2.9±0.3^{ab}	13.4±2.3^{a-f}	1.6±0.3^{ab}
16	196.2±14.4^{ab}	129.2±11.5^{b}	5.4±0.4^{a-d}	27.8±9.5^{ab}	39.6±4.2^{a-d}	2.6±0.3^{a-d}	16.3±4.5^{a-d}	1.8±0.2^{a}
17	208.0±12.0^{ab}	154.6±9.3^{ab}	5.9±0.3^{a-d}	32.6±4.2^{ab}	37.2±6.4^{a-d}	2.8±0.2^{a-d}	13.0±3.1^{b-f}	1.6±0.3^{ab}
18	207.2±26.2^{ab}	145.2±8.2^{ab}	5.9±0.7^{a-d}	26.6±4.6^{ab}	36.0±4.4^{a-d}	2.8±0.4^{a-d}	13.8±1.1^{a-f}	1.5±0.4^{ab}
19	200.8±17.8^{ab}	140.2±11.7^{ab}	5.9±0.3^{a-c}	31.6±4.2^{ab}	36.6±6.2^{a-d}	2.7±0.5^{a-d}	17.6±3.3^{a-c}	1.8±0.4^{a}
20	203.2±10.6^{ab}	157.4±8.7^{ab}	6.1±0.5^{ab}	32.0±7.5^{ab}	39.8±5.7^{a-d}	2.7±0.4^{a-d}	10.6±3.8^{d-f}	1.7±0.4^{a}
21	215.6±12.5^{ab}	137.4±11.9^{ab}	5.9±0.6^{a-c}	34.2±8.2^{a}	35.6±3.8^{a-d}	2.6±0.4^{a-d}	19.8±3.1^{a}	1.7±0.3^{a}
22	199.2±18.1^{ab}	153.8±12.3^{ab}	5.7±0.9^{a-d}	25.0±8.5^{ab}	39.6±6.7^{a-d}	3.1±0.5^{a}	13.0±3.2^{b-f}	1.5±0.2^{ab}
23	209.0±15.4^{ab}	155.6±11.0^{ab}	5.7±0.8^{a-d}	32.4±6.1^{ab}	44.8±9.9^{ab}	2.9±0.2^{a-d}	13. 2±3.7^{b-f}	1.4±0.2^{ab}
24	203.2±6.6^{ab}	151.6±10.6^{ab}	5.9±0.8^{a-d}	29.2±9.9^{ab}	38.8±5.2^{a-d}	2.7±0.3^{a-d}	11.2±1.6^{c-f}	1.5±0.2^{ab}
25	213.8±19.5^{ab}	157.0±9.1^{ab}	5.3±0.8^{a-d}	34.6±4.0^{a}	40.8±3.8^{a-c}	2.8±0.3^{a-d}	12.2±2.6^{c-f}	1.5±0.4^{ab}
26	205.0±21.8^{ab}	149.4±7.5^{ab}	5.7±0.9^{a-d}	27.8±8.5^{ab}	37.4±6.9^{a-d}	2.4±0.3^{de}	9.4±1.8^{f}	1.3±0.4^{ab}
27	194.0±16.2^{b}	143.6±10.2^{ab}	4.8±0.9^{d}	27.4±2.5^{ab}	46.4±3.7^{a}	3.0±0.2^{ab}	15.6±4.2^{a-f}	1.7±0.2^{ab}
28	201.8±13.0^{ab}	148.0±10.3^{ab}	5.1±0.6^{a-d}	33.4±2.2^{a}	42.0±6.7^{a-c}	2.9±0.3^{a-d}	14.0±4.5^{a-f}	1.3±0.4^{ab}
29	206.4±17.0^{ab}	151.8±6.9^{ab}	5.7±0.8^{a-d}	28.6±9.1^{ab}	40.4±6.7^{a-d}	2.9±0.3^{ab}	13.8±2.2^{a-f}	1.3±0.4^{ab}
C	209.2±13.5^{ab}	143.8±13.5^{ab}	5.8±0.8a-d	28.2±8.7^{ab}	35.8±4.6^{a-d}	2.8±0.4^{a-d}	13.2±3.6^{b-f}	1.5±0.4^{ab}

Note: Means in the same columns with different superscripts differ significantly ($p<0.05$). a-c= abc; a-d= abcd; a-e= abcde; a-f= abcdef; a-g= abcdefg; b-d= bcd; b-e= bcde; b-f= bcdef; b-g= bcdefg; c-e= cde; c-f= cdef; c-g= cdefg; d-f= def; d-g= defg.

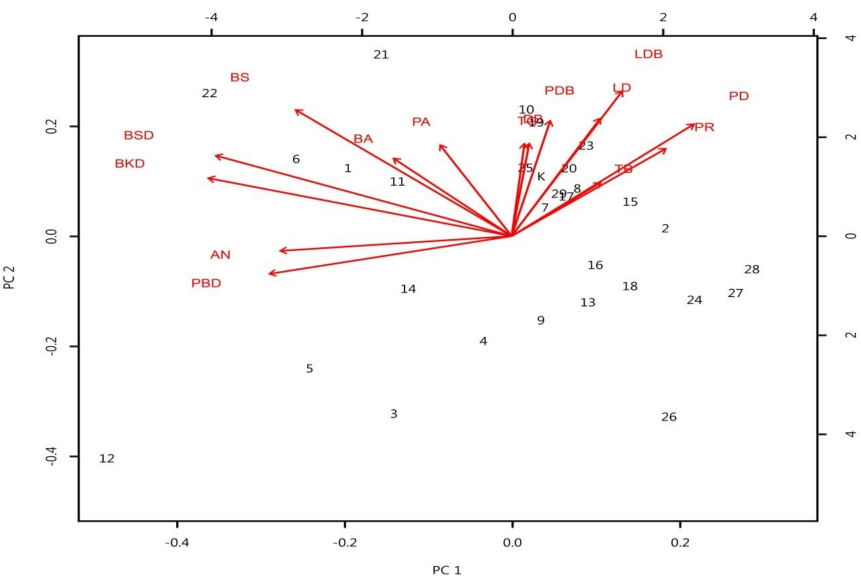

Figure 2. Principal component analysis plots of 30 mutants of Benggala grass based on morphological characters: plant height (TG), forage fresh weight (BS), forage dry weight (DM), leaf width (LD), number of tillers (JA), leaf fresh weight (BSD), leaf dry weight (BKD), percentage of leaf weight (PBSD), root length (PA), and root weight (BA)

Table 4. Average number of tillers, fresh weight, leaf fresh weight, leaf dry weight, percentage of leaf weight, and root length of Benggala grass at 3 months of cutting age

Genotype	Variables					
	Number of tillers	Fresh weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Percentage of leaf weight (%)	Root length (cm)
1	45.0±4.8^{b-d}	1082.0±83.2^{a-d}	369.2±82.8^{a-c}	77.4±9.6^{a-d}	36.1±3.7^{a-c}	43.2±4.9^{a-c}
2	40.0±2.4^{c-d}	914.0±77.0^{a-d}	244.6±40.5^{c-e}	48.8±6.1^{d-g}	28.0±3.6^{c-e}	36.0±5.1^{bc}
3	43.6±2.9^{b-d}	986.0±76.6^{a-d}	273.8±96.9^{c-e}	66.6±10.8^{c-g}	28.6±3.2^{c-e}	39.4±5.4^{a-c}
4	43.0±0.7^{b-d}	794.0±147.2^{b-d}	283.6±67.8^{b-e}	59.0±8.1^{c-g}	33.3±3.7^{b-e}	35.6±4.9^{bc}
5	51.4±1.1^{ab}	890.0±82.8b-^{d}	332.8±91.6^{b-e}	73.2±11.3^{a-f}	38.2±6.1^{ab}	36.0±5.0^{bc}
6	48.6±3.9^{a-d}	1286.0±129.9^{ab}	436.8±42.4^{ab}	87.6±6.1^{a-c}	34.7±4.2^{a-d}	37.4±8.1^{bc}
7	48.8±3.5^{a-c}	930.0±108.9^{a-d}	249.6±69.2^{c-e}	63.0±9.2^{c-g}	27.4±6.5^{c-e}	38.8±6.5^{a-c}
8	45.8±5.6^{b-d}	998.0±156.1^{a-d}	279.0±61.7^{b-e}	55.6±6.2^{d-g}	30.7±8.8^{b-e}	37.2±4.6^{bc}
9	46.4±8.5^{b-d}	794.0±182.7^{b-d}	244.2±85.2^{c-e}	53.4±7.5^{d-g}	31.9±3.3^{b-e}	38.8±7.6^{a-c}
10	44.0±3.4^{b-d}	1132.0±98.8^{a-c}	319.8±73.2^{b-e}	65.8±9.2^{c-g}	27.6±6.4^{c-e}	37.0±4.0^{bc}
11	46.0±4.7^{b-d}	1040.0±142.6^{a-d}	362.6±64.4^{a-d}	71.4±9.6^{b-f}	36.3±6.4^{a-c}	38.6±7.9^{a-c}
12	56.8±2.5^{a}	1090.0±79.1^{a-c}	435.4±90.8^{ab}	98.6±6.2^{ab}	42.3±8.5^{a}	37.4±2.5^{bc}
13	45.0±4.5^{b-d}	996.0±187.2^{a-d}	251.6±57.8^{c-e}	49.8±9.4^{d-g}	26.5±5.3^{de}	38.8±4.6^{a-c}
14	40.2±5.9^{c-d}	998.0±117.8^{a-d}	298.2±73.4^{b-e}	63.28.8±$^{c-g}$	30.1±5.1^{b-e}	43.0±3.4^{a-c}
15	43.4±5.3^{b-d}	1026.0±117.2^{a-d}	243.0±66.8^{c-e}	51.6±7.8^{d-g}	24.5±5.3^{e}	38.0±3.8^{bc}
16	44.8±3.8^{b-d}	722.0±78.55^{cd}	225.6±72.3^{c-e}	47.6±4.8^{d-g}	31.5±7.5^{b-e}	42.0±3.2^{a-c}
17	48.2±4.9^{a-d}	850.0±88.6^{b-d}	257.6±61.0^{c-e}	52.6±8.6^{d-g}	29.9±6.3^{b-e}	41.0±6.9^{a-c}
18	38.6±3.9^{c-d}	732.0±139.9^{cd}	204.4±52.4^{de}	54.0±8.5^{d-g}	32.3±7.8^{b-e}	39.4±2.88^{a-c}
19	42.0±4.0^{b-d}	1020.0±73.8^{a-d}	297.4±40.9^{b-e}	56.4±9.4^{d-g}	29.7±6.9^{b-e}	48.0±4.3^{a}
20	42.4±4.5^{b-d}	1104.0±187.3^{a-c}	305.0±50.0^{b-e}	60.8±8.6^{c-g}	27.8±6.6^{c-e}	37.8±3.6^{bc}
21	47.8±5.5^{a-d}	1290.0±198.4^{ab}	375.0±66.6^{a-c}	75.4±4.2^{a-e}	30.9±5.9^{b-e}	39.0±5.9^{a-c}
22	47.6±4.7^{a-d}	1400.0±175.1^{a}	450.0±73.9^{a}	101.2±9.0^{a}	38.7±4.8^{ab}	41.6±3.4^{a-c}
23	45.4±5.0^{b-d}	1010.0±160.3^{a-d}	265.0±50.3^{c-e}	56.6±14.1^{d-g}	27.2±2.8^{c-e}	40.4±7.5^{a-c}
24	38.8±5.0^{c-d}	678.0±70.5^{cd}	200.6±54.0^{de}	43.2±7.5^{e-g}	30.7±3.3^{b-e}	37.4±4.2^{bc}
25	45.2±4.0^{b-d}	1106.0±133.3^{a-c}	312.2±68.8^{b-e}	65.8±11.9^{c-g}	29.4±3.9^{b-e}	38.0±2.4^{bc}
26	44.4±6.2^{b-d}	672.0±97.6^{cd}	180.6±53.6^{e}	35.4±7.1^{g}	27.6±5.1^{c-e}	33.8±3.8^{c}
27	37.8±1.3^{d}	582.0±194.4^{d}	197.2±74.1^{e}	45.0±7.5^{e-g}	32.8±5.7^{b-e}	34.0±2.6^{c}
28	40.2±3.7^{c-d}	770.0±197.6^{cd}	188.6±69.8^{e}	45.4±8.3^{e-g}	24.7±4.1^{e}	37.0±5.7^{bc}
29	41.8±3.3^{b-d}	1048.0±155.5^{a-d}	292.6±56.8^{b-e}	61.2±5.1^{c-g}	27.1±1.8^{c-e}	40.0±6.1^{a-c}
C	38.6±2.8^{c-d}	1052.0±148.9^{a-d}	272.6±32.5^{c-e}	60.0±10.2^{c-g}	27.0±1.9^{c-e}	44.4±3.3^{ab}

Note: Means in the same columns with different superscripts differ significantly ($p<0.05$). a-c= abc; a-d= abcd; a-e= abcde; a-f= abcdef; a-g= abcdefg; b-d= bcd; b-e= bcde; b-f= bcdef; b-g= bcdefg; c-e= cde; c-f= cdef; c-g= cdefg; d-f= def; d-g= defg.

The analysis results using *heatmap clustering* showed the relationship between morphological characteristics and plant characteristics (Figure 3). Benggala grass mutants were divided into 2 major groups. The first group consisted of 2 sub-groups, which showed the characteristics of fresh weight, leaf fresh, and dry weight, relatively high number of tillers, including numbers 22, 11, 21, 12, and 1. The second group, divided into three subgroups, had low average of fresh forage weight, fresh leaf weight, dry leaf weight, and the number of tillers.

Analysis of Forage Nutritional Quality

The results showed that the nutrient content of the forage of Benggala grass was influenced by gamma-ray irradiation. The contents of forage nutrients such as crude protein, dry matter digestibility, organic matter digestibility, and energy increased, while the crude fiber content decreased in the mutant plants compared to controls (Table 7). An increase in forage quality occurred in mutant number 13, and the crude protein content increased to 9.27%, as well as the dry matter digestibility increased to 79.93%, while the crude fiber decreased from 32.32%.

DISCUSSION

The productions of fresh and dried forages are the direct character of economic value and indicate Benggala grass productivity (Stida *et al.,* 2018). Since this characteristic is the main indicator in determining the productivity of Benggala grass, the forage weight and dry weight are the characteristics that must be considered, other than indirect characters such as the number of tillers, plant height, stem diameter, and other characteristics.

The increased production using mutation breeding with gamma rays also occurs in soybean (Mudibu *et al.,* 2012), feed sorghum (Singh *et al.,* 2013), and okra (Amir *et al.,* 2018). Plant biomass increases due to mutations were found in *Digitaria exilis* (Animasaun *et al.,* 2014)

Table 5. Genetic and phenotypic variants, heritability, and variance coefficients of genetic and phenotypes of Benggala grass

No	Character	Indicators						
		σ^2g	σ^2f	h^2 (%)	*VCG	**VCP	$2\ \sigma_{\sigma^2 G}$	Criteria σ^2g
Cutting age of 2 months								
1	Plant height	28.89	86.62	33.35	2.73	4.72	46.5	narrow
2	Stem height	23.36	73.88	31.62	3.48	6.2	39.77	narrow
3	Number of tiller	19.55	34.3	57	14.38	19.05	17.84	broad
4	Stem diameter	0.09	0.22	42.45	5.27	8.09	0.11	narrow
5	Internode length	2.21	5.54	39.87	4.98	7.89	2.43	narrow
6	Leaf length	9.16	18.8	48.73	6.52	9.34	9.88	narrow
7	Leaf width	0.01	0.02	20.5	2.93	6.48	0.01	narrow
8	Flag leaf length	4.19	7.05	59.38	16.42	21.31	3.66	broad
9	Flag leaf width	0.02	0.05	31.91	10.38	18.38	0.03	narrow
10	Fresh weight	11832	18934	62.49	12.04	15.23	9794	broad
11	Dry weight	76.69	830.76	9.23	5.32	17.49	465.46	narrow
12	Root length	1.13	8.15	13.84	3.36	9.04	4.52	narrow
14	Fresh leaf weight	2288	4168	54.89	19.52	26.35	2173	broad
15	Dry leaf weight	12.45	121.92	10.21	6.28	19.65	68.18	narrow
16	% Leaf weight	23.79	56.22	42.32	4.88	7.5	23.21	broad
Cutting ages of 3 months								
1	Stem height	22.42	98.2	22.83	3.24	6.78	53.65	narrow
2	Number tiller stem	70.73	17.38	44.48	9.98	17.2	6.59	broad
3	Diameter	0.04	0.16	24.78	3.56	7.15	0.09	narrow
4	Internode length	1.46	10.2	14.29	4.08	10.2	4.46	narrow
5	Leaf length	4.42	18.9	23.39	5.6	11.57	10.31	narrow
6	Leaf width	0.02	0.04	50.89	6.1	8.55	0.02	narrow
7	Flag leaf length	2.54	6.16	41.24	11.54	17.98	3.27	narrow
8	Fresh weight	16163	37037	43.64	13.16	19.91	19584	narrow
9	Dry weight	172.32	365.32	47.17	20.07	29.23	165.11	broad
10	Fresh leaf weight	2846	4220	67.44	22.11	26.92	2173	broad
11	% Leaf weight	11	18.3	60.08	10.77	13.9	9.49	broad
12	Dry leaf weight	153.8	234.99	65.45	20.16	24.92	121.23	broad narrow
13	Root length	1.79	9.35	19.18	3.44	7.84	5.14	

Note: σ2g= genetic variants; σ2f= phenotypic variants; h2= heritability; *VCG= variance coefficients of genetic; **VCP = variance coefficients of phenotypes; σσ2g= standard deviation of genetic variance.

Table 6. Relationship between characters in Benggala grass and the principal components 1, 2, 3, and 4

Variables	Principal components			
	1	2	3	4
Plant height	-0.04	0.167	0.847	0.001
Stem height	0.023	0.088	0.857	0.219
Stem diameter	0.071	0.173	0.025	0.651
Internode length	-0.4	0.534	0.423	-0.071
Leaf length	0.017	0.639	-0.133	0.375
Leaf width	-0.222	0.017	0.145	0.821
Flag leaf length	-0.086	0.915	0.171	0.062
Flag leaf width	-0.051	0.881	0.134	0.011
Number of tillers	0.705	-0.248	-0.204	-0.368
Fresh weight	0.804	0.344	0.12	0.209
Fresh leaf weight	0.894	-0.255	-0.066	-0.047
Dry leaf weight	0.906	-0.254	-0.012	-0.132
% Leaf weight	0.324	-0.612	-0.233	-0.307
Root weight	0.624	0.033	0.54	-0.019

and wild ginseng (Le *et al.,* 2019). Plant production is a complex trait, making it difficult to increase yield potential in plants that are previously the result of intensive breeding. However, for plants generated by non-intensive breeding, such as forages, potential yield can be improved by developing new ideotypes through mutation and modifying plant architecture (Shu *et al.,* 2012).

The effect of gamma-ray irradiation on the nutritional content of forage plants was reported by Mohajer *et al*. (2014) in *Onobrychis viciifolia Scop* that crude protein content, dry matter digestibility, Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF) were affected by gamma-ray radiation. Crude protein and dry matter digestibility increased in irradiated plants, while the content of crude fiber, ADF, and NDF decreased.

Mutation induction can reduce lignin and increase crude protein in mutant sorghum (Wahyono *et al.,* 2019). Crude protein content in forage is a positive indicator of the quality of forage plants. Protein is required for plant organ growth, development, reproduction, and repair. Low protein forages can limit livestock performance, whereas high protein forages will usually have high

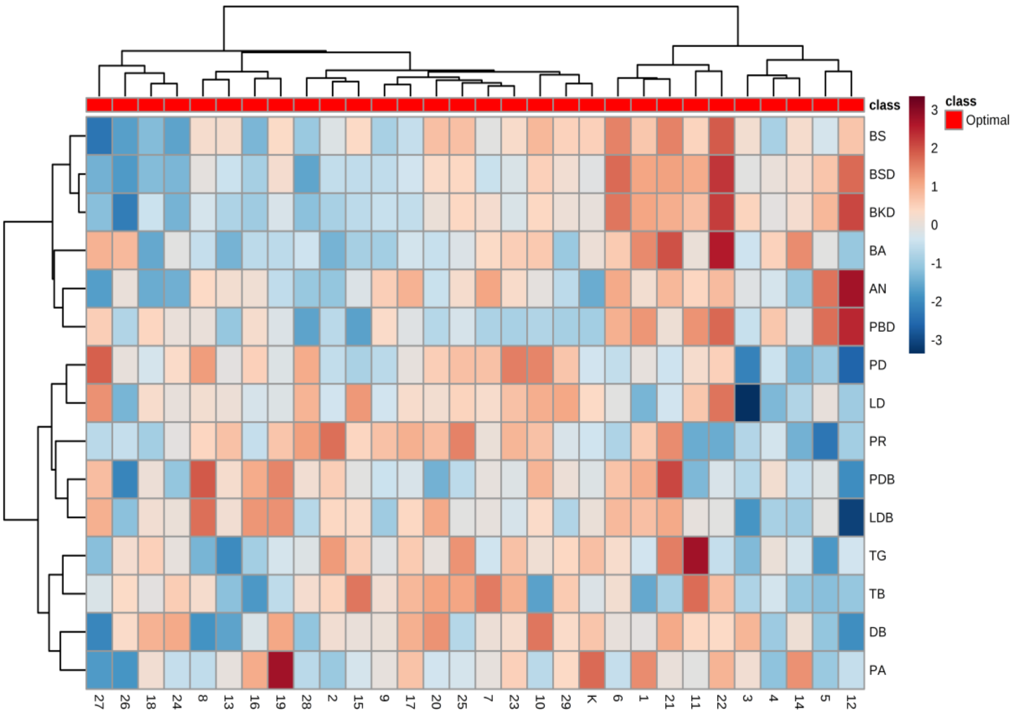

Figure 3. Dendrogram of results of cluster analysis on morphological characters of 30 Benggala grass mutants based on the degree of similarity percentage of plant height (TG), forage fresh weight (BS), forage dry weight (DM), leaf width (LD), number of tillers (JA), leaf fresh weight (BSD), leaf dry weight (BKD), percentage of leaf weight (PBSD), root length (PA), root weight (BA). Note: The higher red intensity the higher rate, the higher the blue intensity the lower rate.

Table 7. The results of the analysis of crude protein, crude fiber, energy, dry matter digestibility, and organic matter digestibility of mutants of Benggala grass

Genotype	Variables				
	Crude protein (%)	Crude fiber (%)	Energy (J)	Dry matter digestibility (%)	Organic matter digestibility (%)
1	8.17^{ab}	38.12^{bcd}	3753.50^{bcde}	79.93^{a}	78.90^{a}
2	7.88^{ab}	32.32^{e}	3702.50^{de}	78.11^{a}	75.55^{ab}
5	8.69^{ab}	38.58^{bcd}	3878.00^{a}	68.74^{bcd}	67.21^{cdef}
6	8.74^{ab}	39.38^{bc}	3652.00^{e}	57.35^{e}	54.45^{g}
7	8.47^{ab}	38.11^{bcd}	3662.50^{de}	73.31^{abc}	74.86^{abc}
10	8.11^{ab}	34.48^{ed}	3671.50^{de}	68.57^{bcd}	66.90^{cdef}
11	7.68^{ab}	39.13^{bc}	3662.00^{de}	75.57^{ab}	74.39^{abc}
12	7.55^{b}	37.65^{cd}	3734.50^{cde}	69.94^{bcd}	67.87^{bcdef}
13	9.27^{a}	38.11^{bcd}	3708.50^{de}	73.31^{abc}	71.55^{abcde}
14	7.94^{ab}	34.56^{ed}	3850.50^{ab}	68.38^{bcd}	66.87^{cdef}
15	7.87^{ab}	39.74^{bc}	3850.00^{ab}	74.25^{abc}	73.29^{abcd}
17	7.39^{b}	38.19^{bcd}	3830.50^{abc}	67.35^{cd}	66.06^{def}
20	8.94^{ab}	34.75^{ed}	3658.00^{de}	68.23^{bcd}	65.11^{ef}
21	7.64^{b}	42.18^{b}	3708.00^{de}	77.71^{a}	74.93^{abc}
22	8.23^{ab}	37.16^{cd}	3770.00^{abcd}	79.12^{a}	78.01^{a}
23	7.49^{b}	38.18^{bcd}	3765.50^{abcde}	69.97^{bcd}	69.11^{bcdef}
Control	7.55^{b}	46.43^{a}	3823.00^{abc}	65.58^{d}	62.79^{f}

Note: Means in the same columns with different superscripts differ significantly ($p<0.05$)

energy and are good for livestock performance, where crude protein will positively correlate with digestibility of organic matter and negatively correlate with crude fiber (Zhang *et al.*, 2018). The increase in protein in Benggala grass caused by gamma-ray radiation in this study suggests that breeding by gamma-ray mutation can be utilized to improve the nutritional content of forages.

Crude fiber, particularly the high level of NDF in forages, will restrict intake and decrease the efficacy of forage digestion. In addition to NDF in crude fiber, ADF in forages negatively correlates with digestibility

(Zhang *et al.,* 2018). The results showed that the mutants had lower crude fiber content than the control, and the reduction in crude fiber was expected to improve the forage's quality and digestibility.

Gamma rays are assumed to improve the nutritional content of forage by stimulating growth via modifying hormones that affect plant cells. Furthermore, low-intensity gamma rays will improve the ability of the antioxidants in the cell to overcome stress conditions (Moghaddam *et al.,* 2011). Beyaz *et al*. (2016) reported an increase in catalase activity (CAT) and total chlorophyll in sainfoin plants (*Onobrychis viciifolia* Scop) that was irradiated by gamma rays. Besides, CAT activity also increased in Arbidopsis, which was irradiated with 50 Gy gamma rays (Qi *et al.,* 2014) and *Brachypodium distachyon* that was irradiated with 150 Gy (Kim *et al.,* 2015).

Principal component analysis (PC) can classify the mutants of Benggala grass based on morphological characteristics that affect production. Principal component 1 (Table 6) shows the characteristics directly related to the mutant production of Benggala grass are the number of tillers, fresh weight of forage, dry leaf weight, and root weight. They are commonly used to measure the production of Benggala grass. Principal component analysis 2 shows more specific characteristics because the characteristics related to this PC are internode length, leaf length, width and length of the flag leaf, which can be used as character traits for a genotype, such as a flag leaf characteristic. The flag leaf characteristic is associated with the production of cereal crops (Al-Tahir, 2014). In addition, flag leaves are also related to the developmental metabolites of a plant (Hu *et al.,* 2020). The grouping of plants on PC2 based on specific characteristics is expected to focus more on specific genotypes/clones to be selected as superior clones. The results of grouping plants using cluster analysis (Figure 3) show that mutant clones are divided into two large groups based on the character of plant production. Mutant clones associated with PC 1 and PC2 clustered in plants with high forage production characteristics. The clones that have the potential to become superior clones based on grouping using principal component analysis and cluster analysis are the clone numbers 6, 1, 21, 11, and 22. These clones have character traits associated with high production, so they can be developed into superior clones. The grouping of superior genotypes using PCA has also been successfully carried out. The PCA has been used to investigate the production and qualities that impact sweet potato parents (Rukundo *et al.,* 2015), vertiver grass (*Vertiver zizanioides* L.) (Lal *et al.,* 2018), maize (*Zea mays*), and nutritional qualities (Magudeeswari *et al.,* 2019).

This study indicates that gamma-ray irradiation can improve the morphological characteristics and nutritional values of Benggala grass. Therefore, it is expected to produce superior Benggala grass to meet the forage needs of farmers.

CONCLUSION

Selection in the M1V3 generation produced 5 Benggala grass mutants with higher production than the control variety. Mutants in the M1V3 generation also had better nutritional values than control varieties. In the M1V3 generation, genetic parameters were also obtained with broad categories on the number of tillers, fresh weight and leaf width, which can be used as selection characters in the next generation to produce Bengal grass varieties with high productivity.

CONFLICT OF INTEREST

There is no conflict of interest in any financial, personal, or other relationship with other people or organizations related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

Thank you to the agrostology staff of the Indonesian Research Institute of Animal Production (IRIAP) Ciawi-Bogor, who assisted in field and laboratory observations. Our gratitude also goes to Dr. Waras Nurcholis, who has helped us in data analysis, and our thanks go to the Agricultural Research and Development Agency for funding this research.

REFERENCES

Aisyah, S. I., H. Aswidinnoor, A. Saefuddin, B. Marwoto, & S. Sastrosumarjo. 2009. Induksi mutasi pada setek pucuk anyelir (*Dianthus caryophyllus* Linn.) melalui iradiasi sinar gamma. Jurnal Agronomi Indonesia. 37:62-70.

Amir, K., S. Hussain, M. Shuaib, F. Hussain, Z. Urooj, W. M. Khan, U. Zeb, K. Ali, M. A. Zeb, & F. Hussain. 2018. Effect of gamma irradiation on OKRA 279 (*Abelmoschus esculentus* L.). Acta Ecologica Sinica 38:368–373. https://doi.org/10.1016/j.chnaes.2018.02.002

Animasaun, D. A., J. A. Morakinyo, & O. T. Mustapha. 2014. Assessment of the effects of gamma irradiation on the growth and yield of *Digitaria exilis*. J. Appl. Biosci. 75:6164–6172. https://doi.org/10.4314/jab.v75i1.1

Al-Tahir, F. M. M. 2014. Flag leaf characteristics and relationship with grain yield and grain protein percentage for three cereals. Journal of Medicinal Plants Studies 2:01–07.

Beyaz, R., C. Sancak, Ç. Yildiz, Ş. Kuşvuran, & M. Yildiz. 2016. Physiological responses of the M1 Sainfoin (*Onobrychis viciifolia* Scop) plants to gamma radiation. Applied Radiation and Isotopes 118:73-79. https://doi.org/10.1016/j.apradiso.2016.09.005

Fanindi, A., S. H. Sutjahjo, S. I. Aisyah, & N. D. Purwantari. 2019. Morphological characteristics and productivity of guinea grass (*Panicum maximum* cv purple guinea) irradiated with Gamma-Ray. Trop. Anim. Sci. J. 42:97–105. https://doi.org/10.5398/tasj.2019.42.2.97

Gallo, A., M. Moschini, C. Cerioli, & F. Masoero. 2013. Use of principal component analysis to classify forages and predict their calculated energy content. Animal 7: 930–939. https://doi.org/10.1017/S1751731112002467

Hu, C., J. Rao, Y. Song, S. A. Chan, T. Tohge, & B. Cui. 2020. Dissection of flag leaf metabolic shifts and their relationship with those occurring simultaneously in developing seed by application of non-targeted metabolomics. PLoS ONE 15:1-23. https://doi.org/10.1371/journal.pone.0227577

Kim, D. Y., M. J. Hong, C. S. Park, & Y. W. Seo. 2015. The effects of chronic radiation of gamma ray on protein expression and oxidative stress in *Brachypodium distachyon*. Int. J. Radiat. Biol. 91:407–419. https://doi.org/10.3109/09553002.2015.1012307

Lal, R. K., P. Gupta, & S. Sarkar. 2018. Phylogenetic relationships, path and principal component analysis for genetic variability and high oil yielding clone selection in vetiver (*Vetiveria zizanioides* L.) nash. Journal of Plant Genetics and Breeding 2:105-113.

Le, K. C., T. T. Ho, K. Y. Paek, & S. Y. Park. 2019. Low dose gamma radiation increases the biomass and ginsenoside content of callus and adventitious root cultures of wild ginseng (*Panax ginseng* Mayer). Ind. Crops Prod. 130:16–24. https://doi.org/10.1016/j.indcrop.2018.12.056

Moghaddam, S. S., H. Jaafar, R. Ibrahim, A. Rahmat, M. A. Aziz, & E. Philip. 2011. Effects of acute gamma irradiation on physiological traits and flavonoid accumulation of *Centella asiatica*. Molecules 16:4994–5007. https://doi.org/10.3390/molecules16064994

Mohajer, S., R. M. Taha, M. M. Lay, E. A Khorasani, & M. Khalili. 2014. Stimulatory effects of gamma irradiation on phytochemical properties, mitotic behaviour, and nutritional composition of sainfoin (*Onobrychis viciifolia* Scop.). Scientific World Journal 2014:1–9. https://doi.org/10.1155/2014/854093

Magudeeswari, P., E. V. D. Sastry, & Th. R. Devi. 2019. Principal component (PCA) and cluster analyses for plant nutrient traits in baby corn (*Zea mays* L.). Indian J. Agric. Res. 53:353-357. https://doi.org/10.18805/IJARe.A-5042

Mudibu, J., K. K. C. Nkongolo, A. Kalonji-Mbuyi, & V. Roger. 2012. Effect of gamma irradiation on morpho-agronomic characteristics of soybeans (*Glycine max* L.). Am. J. Plant Sci. 3:331-337. https://doi.org/10.4236/ajps.2012.33039

Pinaria, A., A. Baihaki, R. Setiamihardja, & A. A. Daradjat. 1996. Variabilitas genetik dan heritabilitas karakter katakter biomassa 53 genotip kedelai. Zuriat. 6:88-92.

Qi, W., L. Zhang, H. Xu, L. Wang, & Z. Jiao. 2014. Physiological and molecular characterization of the enhanced salt tolerance induced by low-dose gamma irradiation in *Arabidopsis* seedlings. Biochem. Biophys. Res. Commun. 450:1010–1015. https://doi.org/10.1016/j.bbrc.2014.06.086

Rukundo, P., S. Hussein, L. Mark, & G. Daphrose. 2015. Application of principal component analysis to yield and yield related traits to identify sweet potato breeding parents. J. Trop. Agric. 92:1-15.

Song, J. & B. Li. 2021. Nonlinear and additive principal component analysis for functional data. J. Multivar. Anal. 181:1-21. https://doi.org/10.1016/j.jmva.2020.104675

Singh, R. K. & B. D. Chaundhary. 1977. Biometrical Methods in Quantitative Genetics Analysis. New Delhi, Kalyani Publishers.

Singh, B., S. Ahuja, R. K. Singhal, & P. V. Babu. 2013. Radiosensitivity studies and radiostability of ribulose-1,5 bis-carboxylase and gas exchange characteristics in wheat, garden pea, field pea, spinach, and okra. Water Air Soil Pollut. 225:1-8. https://doi.org/10.1007/s11270-013-1815-7

Stida, W. F., R. F. Daher, A. P. Viana, A. K. F. Vidal, R. S. Freitas, V. B. da Silva, A. V. Pereira, S. Cassaro, B. R. da S. Menezes, & E. P. Furlani. 2018. Estimation of genetic parameters and selection of elephant-grass (*Pennisetum purpureum* Schumach.) for forage production using mixed models. Chil. J. Agric. Res. 78:198-204. https://doi.org/10.4067/S0718-58392018000200198

Shu, Q., B. Forster, & H. Nakagawa. 2012. Principles and Applications of Plant Mutation Breeding. In Q. Shu, B. Foster, & H. Nakagawa (Eds.), Plant Mutation Breeding and Biotechnology. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture International Atomic Energy Agency. pp. 301-326. https://doi.org/10.1079/9781780640853.0301

Thiex, N., J. H. Manson, S. Andersson, & J. A Persson. 2002. Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: Collaborative study. J. AOAC Int. 85:309-17. https://doi.org/10.1093/jaoac/85.2.309

Tilley, J. M. A., & R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 18:104-11. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Van Soest, P. J., J. B. Robertson, & B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Wahyono, T., I. Sugoro, A. Jayanegara, K. G. Wiryawan, & D. A. Astuti. 2019. Nutrient profile and *in vitro* degradability of new promising mutant lines sorghum as forage in Indonesia. Adv. Anim. Vet. Sci. 7:810-818. https://doi.org/10.17582/journal.aavs/2019/7.9.810.818

Zen, S. 2012. Parameter genetik padi sawah dataran tinggi. Jurnal Penelitian Pertanian Terapan. 12:196-201.

Zhang, J., A. D. Iwaasa, G. Han, C. Gu, H. Wang, P. G. Jefferson, & J. Kusler. 2018. Utilizing a multi-index decision analysis method to overall assess forage yield and quality of C3 grasses in the western *Canadian prairies*. Field Crops Res. 222:12–25. https://doi.org/10.1016/j.fcr.2018.03.007