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Gamma Irradiation-Induced Changes in Morphology, Nutritional Traits, and *In Vitro* Digestibility of *Pennisetum purpureum* cv. Mott on Post-Gold Mining Soil

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ABSTRACT

Post-mining lands, especially abandoned gold mining areas, present serious challenges for agriculture and forage cultivation due to poor soil fertility, heavy metal contamination, and damaged soil structure. To address these issues, this study evaluated the impact of gamma irradiation on the agronomic traits, nutritional content, anatomical characteristics, and *in vitro* digestibility of dry matter, organic matter, and fiber fractions of *Pennisetum purpureum* cv. Mott grown on degraded post-gold mining soil. A completely randomized design was used, applying seven doses of gamma rays (0, 5, 10, 15, 20, 25, and 30 Gy). Initially, irradiated plants were cultivated under optimal soil conditions. Selected cuttings were then transplanted onto post-mining soil for two months. Variables observed included growth performance, proximate composition, fiber fractions, macro minerals, and digestibility (dry matter digestibility [DMD], organic matter digestibility [OMD], *in vitro* digestibility [IVD], neutral detergent fiber digestibility [NDFD], acid detergent fiber digestibility [ADFD], cellulose digestibility [CeD], hemicellulose digestibility [HmD]). Results showed that a moderate dose of 10 Gy significantly enhanced dry matter digestibility, *in vitro* digestibility, and fiber degradation, accompanied by increases in crude protein and mineral levels. Anatomically, beneficial changes such as thicker mesophyll and vascular tissues were observed at this dose. While the 20 Gy dose also showed improvements in some fiber digestibility parameters, it did not provide an optimal balance with productivity, which was better achieved at 10 Gy. Multivariate analysis revealed distinct treatment clustering, reflecting physiological responses to irradiation. Cultivars treated with 10 Gy exhibited an optimal balance between productivity and forage quality. These findings suggest that gamma irradiation can successfully induce advantageous mutations, improving both adaptability and nutritional value of *P. purpureum* on marginal soils. In particular, a dose of 10 Gy is promising for breeding superior forage cultivars for the reclamation of post-mining land.

Keywords: forage quality; gamma irradiation; land reclamation; Pennisetum purpureum; post-mining land

INTRODUCTION

The reclamation and sustainable use of post-mining lands have become an urgent priority due to accelerating soil degradation and ecosystem disruption caused by extractive industries (Sihombing *et al*., 2025). Gold mining, particularly in tropical regions such as Indonesia, has led to severe environmental problems, including the deterioration of soil structure, nutrient depletion, and heavy metal contamination (Kaba *et al*., 2025). These conditions restrict natural vegetation recovery and limit opportunities for agricultural and pastoral development (Sun *et al*., 2024). Identifying forage species that can tolerate such adverse environments while providing ecological and economic value is therefore essential for reclamation programs (Putra *et al*., 2025a). Such efforts are expected to restore ecosystem services and support food and feed security in mining-affected landscapes.

Pennisetum purpureum Schumach., commonly referred to as Napier grass, is a perennial C_4 species valued for its high biomass production and strong adaptability to tropical environments (Aleme *et al*., 2024). The dwarf cultivar 'Mott' is widely used as forage because of its higher leaf-to-stem ratio and improved digestibility. However, its performance is markedly reduced under the harsh edaphic conditions of post-mining soils (Putra *et al*., 2022a). Induced mutagenesis has emerged as a promising strategy to enhance its resilience and nutritional quality. Gamma irradiation, in particular, has been extensively applied in plant breeding and is reported to improve vigor, nutrient content, and stress tolerance (Al-Sayed *et al*., 2025; Putra *et al*., 2025b). Nevertheless, little attention has been given to its potential for improving forage crops cultivated on gold mine-degraded soils.

Despite the potential benefits of induced mutagenesis, there is a notable lack of research on its use to improve forage crops specifically for cultivation on post-mining land. Moreover, studies that integrate a comprehensive analysis of agronomic, nutritional, and anatomical properties, combined with multivariate analysis, to assess irradiated genotypes under these unique stress conditions are limited. The novelty of this study lies in being the first to integrate agronomic, nutritional, anatomical, and digestibility traits of gamma-irradiated *P. purpureum* cv. Mott cultivated on degraded gold mining soil, analyzed through a multivariate framework. This research fills that gap by providing a holistic evaluation of the effects of gamma irradiation on a promising forage cultivar grown on gold mine-degraded soil.

This study aims to evaluate the performance of gamma-irradiated *Pennisetum purpureum* cv. Mott cultivated on degraded gold mine soil. Specifically, it examines key growth traits, nutritional composition, fiber fractions, and digestibility parameters. By utilizing a comprehensive multivariate approach, this study seeks to identify mutant lines with superior adaptation and forage quality. The findings are expected to contribute to the development of improved forage cultivars suitable for land reclamation and sustainable livestock systems on degraded landscapes.

MATERIALS AND METHODS

Study Area and Experimental Period

The study was conducted in Kota Baru Santan, Tubei sub-district of Lebong Regency, Bengkulu Province, Indonesia. Geographically, the site is located at -3.1667240 latitude and 102.1432690 longitude, with an elevation of 97 meters above sea level. A detailed map illustrating the exact location is presented in Figure 1. The experimental period covered two months of plant growth in post-gold mining soil after an initial two-month acclimatization phase in optimal soil conditions.

Gamma Irradiation Process

In this study, gamma irradiation was applied directly to stem cuttings of *Pennisetum purpureum* cv. Mott, which served as the experimental material. This approach deviates from conventional practices that typically use seeds or generative plant materials, and it was chosen due to the limited availability of *P. purpureum* cv. Mott seeds. The aim was to evaluate the efficacy of mutation induction through vegetative organs to maximize the adaptive potential of gamma irradiation in genetic modification. All irradiation procedures were conducted at the BATAN facility (National Nuclear Energy Agency, Indonesia). Stem cuttings of *P. purpureum* cv. Mott was irradiated using a Cobalt-60 radiation source in a Gamma Cell 4000A irradiator at a dose rate of 0.0775 rad/second. Acute doses administered included 0 Gy (unirradiated control), 5, 10, 15, 20, 25, and 30 Gy. Each dose variation was applied to 100 stems, resulting in a total of 1,100 irradiated samples and 100 control stems. Radiosensitivity testing in the first generation (M1) revealed a 50% lethal dose (LD_{50}) of 27 Gy, as determined

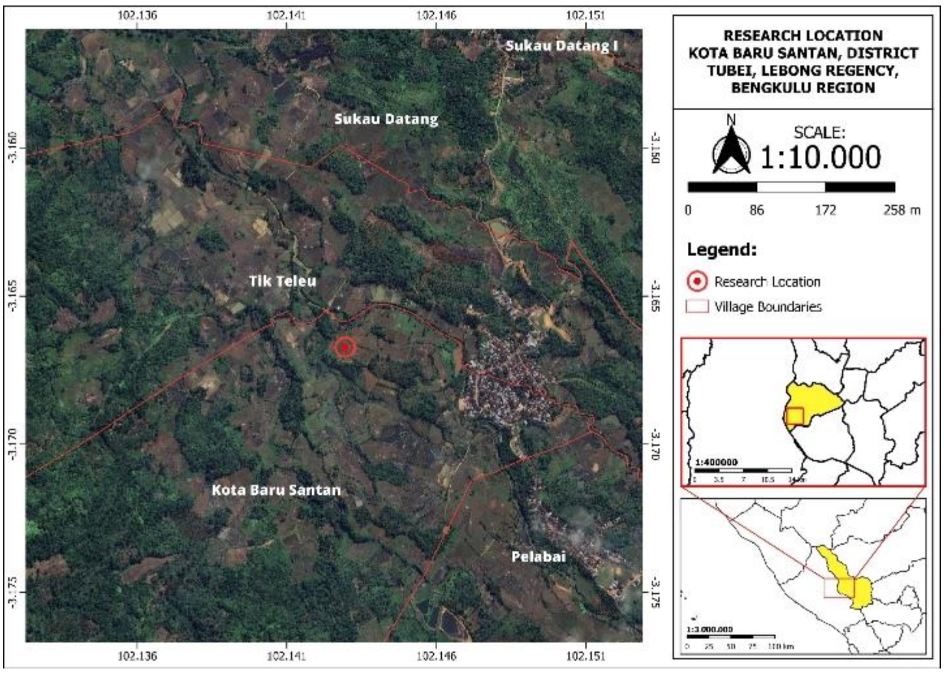

Figure 1. Map of the study area in Kota Baru Santan, Lebong Regency, Bengkulu Province, Indonesia, highlighting the exact geographical coordinates of the experimental site.

by probit analysis of germination percentage data from 100 cuttings per dose. Detailed morphological performance of the M1 generation—including leaf count, leaf length, leaf width, plant height, stem diameter, stem fresh weight, root length, and root fresh weight—has been reported in our previous study (Putra & Prasetya, 2024), and serves as the baseline reference for evaluating subsequent generations in this research.

Plant Cultivation and Experimental Design

The study began with the irradiation of *P. purpureum* cv. Mott stem cuttings. Following irradiation, the cuttings were initially cultivated for two months directly in normal (non-degraded) soil to ensure uniform early growth and acclimatization under optimal conditions. During this initial phase, plants received standard NPK fertilizer (16:16:16) at a dose of 5 g per plant, applied every two weeks to support robust initial development. The plants were harvested after this initial growth period, and second-generation stem cuttings were taken for further cultivation.

These second-generation cuttings were then transplanted into polybags measuring 40 cm x 40 cm containing degraded post-gold mining soil, which was collected from Lebong Village, Bengkulu Regency, Indonesia. The soil used in this study has been previously analyzed and reported in Putra *et al*. (2022b). The results of the soil analysis revealed the following properties: Cd = 0.173 mg kg^{-1}, Hg = 0.635 mg kg^{-1}, Cr = 0.8 mg kg^{-1}, Fe = 1.16%, Al_2O_3 = 5.09%, water content = 34.2%, pH = 4.90, total N = 0.141%, available P = 3.14%, and exchangeable K = 0.052%. The post-mining soil used in this study exhibited poor physical structure, low nutrient availability, and traces of heavy metal contamination. To assess the natural adaptability of the irradiated plants, no fertilizers were applied during the two-month cultivation period. The trial was arranged outdoors using polybags and followed a completely randomized design (CRD), where gamma irradiation dose served as the only treatment factor. Treatments were randomly distributed among polybags, with four replications for each dose to strengthen the reliability of the analysis. This design provided a clear framework for evaluating growth responses and tolerance of the mutants under the stressful edaphic conditions typical of degraded post-mining land.

Agronomic Parameters Measurement

Agronomic observations were taken two months after transplanting into the post-mining soil. The recorded traits covered plant height, leaf dimensions (length and width), number of leaves, fresh and dry weights of leaves, stem length, number of tillers, stem diameter, number of nodes, internode length, and both fresh and dry stem weights.

Chemical Composition Analysis

Plant samples of *P. purpureum* cv. Mott was harvested after two months of growth in polybags. The above-ground biomass, consisting of leaves and stems, was pooled and analyzed for proximate composition. Parameters measured included moisture content, crude lipid (ether extract, EE), ash, crude fiber (CF), and crude protein (CP), using Near-Infrared Reflectance Spectroscopy (NIRS) following the procedure of Rambo *et al*. (2020). The contents of acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to Van Soest *et al*. (2020), which applies mild detergents to remove proteins and hemicellulose. Cellulose concentration was obtained by subtracting lignin from ADF, and the value was reported as crude cellulose (Navarro *et al*., 2018).

In Vitro Digestibility Analysis

In vitro digestibility was evaluated using a modified method derived from Tilley and Terry (1963), with the Daisy II incubator system (ANKOM Technology) applied as described by Tassone *et al*. (2020). Rumen fluid was collected from fistulated Peranakan Ongole (PO) cattle, an Indonesian local crossbred derived from Ongole cattle, maintained at the BRIN research facility in Cibinong, Bogor, Indonesia. The cattle had been previously fistulated and were routinely maintained by BRIN staff; no surgical procedure was performed specifically for this study. The use of these animals complied with the ethical standards for animal care established by BRIN and followed standard procedures for animal welfare (Bayne, 1998). Preparation of the rumen inoculum and buffer solutions followed the protocol outlined by Aguerre *et al*. (2023). The digestibility parameters measured included dry matter digestibility (DMD), organic matter digestibility (OMD), *in vitro* digestibility (IVD), neutral detergent fiber digestibility (NDFD), acid detergent fiber digestibility (ADFD), cellulose digestibility (CeD), and hemicellulose digestibility (HmD).

Statistical Analysis

The collected data were subjected to analysis of variance (ANOVA) based on a completely randomized design (CRD) with seven treatments and four replicates. Duncan's Multiple Range Test (DMRT) was applied to identify significant differences among treatments and support a robust interpretation of experimental outcomes. In addition to univariate analysis, multivariate analysis was employed to provide a more comprehensive evaluation of the data, including identifying treatment clustering and relationships among variables. All statistical analyses were performed using R statistical software.

RESULTS

Leaf Morphological

Gamma irradiation produced significant effects on several leaf-related traits (Table 1). Plant height, leaf fresh weight, and leaf dry weight showed clear treatment differences (p<0.01), while leaf length, width, and number did not vary significantly (p>0.05). The most pronounced responses appeared at low irradiation levels, where plants tended to be taller and produced greater leaf biomass compared with the control. In contrast, higher irradiation levels (15–30 Gy) were associated with reduced growth. Leaf length, width, and number showed small numerical variation among treatments but no consistent trends.

Stem Morphological

Stem traits also showed varied responses to irradiation (Table 2). Fresh and dry weights were strongly affected (p<0.01), and internode length displayed moderate differences among treatments (p<0.05). Other parameters, such as stem length, number, and diameter, were not significantly influenced (p>0.05). Low-dose treatments (5–10 Gy) supported greater stem biomass and longer internodes compared with higher-dose groups. Once irradiation reached 15 Gy or above, both stem fresh and dry weights declined, and internodes tended to be shorter.

Chemical Composition

Analysis of proximate composition revealed limited effects of irradiation (Table 4). Significant differences were found only for dry matter content (p<0.05), while

Table 1. Morphological characteristics of *Pennisetum purpureum* cv. Mott leaves exposed to different doses of gamma irradiation

Treatments	Leaf morphological			
	Plant height (cm)	Leaf length (cm)	Leaf width (mm)	Leaf number
Un irradiated	105.88±8.64^{a}	71.38±1.60	39.31±2.90	182.00±23.93
IR 5	110.67±7.54^{a}	73.50±7.19	39.78±7.79	244.75±72.15
IR 10	107.33±8.26^{a}	66.25±6.50	40.57±2.82	274.00±18.67
IR 15	89.50±7.59^{b}	65.25±3.69	36.40±2.71	252.00±51.43
IR 20	86.50±4.51^{b}	65.75±4.19	37.96±1.42	213.00±33.91
IR 25	91.75±6.99^{b}	63.00±5.72	35.39±4.60	226.33±66.96
IR 30	89.75±5.91^{b}	64.00±4.24	36.46±1.53	210.75±35.61
p	<0.01	>0.05	>0.05	>0.05

Note: Values represent the mean ± standard deviation. Different superscript letters within the same column indicate significant differences among treatments at p<0.01 according to Duncan's multiple range test. Gamma irradiation treatments included 0 Gy (unirradiated control) and doses of 5, 10, 15, 20, 25, and 30 Gy. Morphological traits measured included plant height, leaf length, leaf width, number of leaves, leaf fresh weight, and leaf dry weight. Low-dose irradiation (5–10 Gy) generally enhanced growth parameters, while higher doses (≥15 Gy) negatively impacted plant morphology.

Table 2. Stem morphological characteristics of *Pennisetum purpureum* cv. Mott exposed to different doses of gamma irradiation

Treatments	Stem morphological				
	Stem length (cm)	Stems number	Stem diameter (mm)	Nod number	Nod length (mm)
Un irradiated	34.50±7.72	16.75±6.60	18.84±4.13	19.00±3.56	17.01±2.61^{a}
IR 5	33.00±9.87	18.50±6.45	23.70±2.82	15.50±7.94	18.55±2.73^{ab}
IR 10	32.25±14.50	22.50±1.73	22.49±1.66	14.25±7.76	22.05±4.68^{b}
IR 15	24.25±4.35	20.00±4.08	22.60±0.99	9.00±2.71	19.26±2.89^{ab}
IR 20	20.75±5.56	17.50±1.29	24.63±2.99	11.50±3.42	15.09±2.01^{a}
IR 25	28.75±8.54	16.25±8.42	21.67±0.97	13.75±7.41	14.67±1.56^{a}
IR 30	25.75±6.18	17.00±3.46	22.06±2.87	9.25±2.22	15.39±3.79^{a}
p	>0.05	>0.05	>0.05	>0.05	<0.05

Note: Data are presented as mean ± standard deviation. Different letters within the same column indicate significant differences at p<0.05 according to Duncan's Multiple Range Test (DMRT). Treatments included unirradiated control (0 Gy) and gamma irradiation doses of 5, 10, 15, 20, 25, and 30 Gy. Traits observed were stem length, stems number, stem diameter, nodes number, and node length.

Table 3. Biomass production and leaf/stem ratio of *Pennisetum purpureum* cv. Mott under different doses of gamma irradiation

Treatment	Stem (g)		Leaf (g)		Total (g)		Leaf stem ratio	
	FW	DW	FW	DW	FW	DW	FW	DW
Un irradiated	1695.20±276.31^{bc}	264.71±56.76^{a}	942.36±121.72^{a}	192.96±15.76^{ab}	2637.56±397.7^{ab}	457.67±72.1^{ab}	0.55±0.02	0.72±0.01
IR 5	3217.87±334.42^{a}	517.39±55.68^{b}	1694.57±300.38^{b}	346.62±52.35^{c}	4912.43±589.8^{c}	864.01±70.9^{c}	0.52±0.06	0.66±0.13
IR 10	3056.59±241.21^{a}	472.01±30.07^{b}	1737.25±103.13^{b}	336.21±21.07^{c}	4793.84±314.2^{c}	808.22±33.1^{c}	0.56±0.04	0.71±0.06
IR 15	1777.26±710.08^{b}	267.81±91.85^{a}	1139.53±186.25^{a}	244.72±43.02^{a}	2916.78±847.3^{b}	512.53±122.2^{b}	0.64±0.28	0.97±0.32
IR 20	1740.75±86.00^{bc}	286.21±28.62^{a}	998.79±123.72^{a}	186.09±28.10^{b}	2739.54±203.5^{ab}	472.3±38.2^{ab}	0.57±0.05	0.65±0.12
IR 25	1651.10±831.62 ^{bc}	262.31±119.64^{a}	880.56±424.42^{a}	168.27±80.42^{b}	2531.66±1251.4^{ab}	430.58±197.9^{ab}	0.53±0.06	0.64±0.1
IR 30	1040.38±67.75 ^{bc}	194.08±24.90^{a}	781.50±171.49^{a}	148.81±55.10^{b}	1821.87±194.8^{a}	342.89±66.5^{a}	0.75±0.16	0.76±0.26
p	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	>0.05	>0.05

Note: Data are presented as mean ± standard deviation. Different superscript letters within the same column indicate significant differences at p<0.01 according to Duncan's Multiple Range Test (DMRT). Treatments included unirradiated control (0 Gy) and gamma irradiation doses of 5, 10, 15, 20, 25, and 30 Gy. Measured parameters included stem FW, stem DW, leaf FW, leaf DW, total FW, total DW, and leaf/stem ratios (FW and DW). FW: fresh weight; DW: dry weight.

organic matter, crude fiber, crude protein, ether extract, and total digestible nutrients remained statistically unchanged (p>0.05). Although not significant, DM values were generally higher in plants exposed to low irradiation levels and slightly reduced at higher doses. Some irradiated groups showed minor increases in crude protein and total digestible nutrients, but crude fiber and ether extract were relatively stable across treatments.

Fiber Fraction Content

Gamma irradiation affected fiber fractions to different extents (Table 5). Neutral detergent fiber varied significantly among treatments (p<0.05), while cellulose and hemicellulose contents were highly significant (p<0.01). Lignin fractions, including ADL and total lignin, also showed clear treatment effects (p<0.01). Cellulose levels tended to increase under moderate to high irradiation, whereas hemicellulose declined with rising dose. Lignin accumulation was greatest at higher doses, while the lowest lignin values were consistently recorded in the control plants.

Fiber Fraction Digestibility

Digestibility traits were strongly influenced by irradiation (Table 6). Significant treatment effects (p<0.05) were observed for dry matter digestibility, organic matter digestibility, *in vitro* digestibility, and fiber-related indices such as NDFD and ADFD. The most favorable results were obtained at moderate doses (10–15 Gy), where digestibility values were consistently higher than the control. At doses of 25 Gy and above, digestibility decreased across most parameters. Cellulose and hemicellulose digestibility followed the same trend, with peaks at moderate levels before declining at higher doses.

Multivariate Analysis

Correlation patterns among morphological, chemical, and digestibility traits. The correlation heatmap (Figure 2) illustrated distinct patterns linking morphological performance, proximate composition, fiber fractions, and digestibility traits. Digestibility variables—such as DMD, OMD, IVD, NDFD, and ADFD—were tightly interconnected and formed a coherent cluster in the hierarchical analysis. These traits were also positively aligned with cell wall constituents, particularly cellulose and hemicellulose, suggesting that improvements in these structural carbohydrates supported enhanced digestibility.

On the other hand, lignin-related parameters, including ADL and total lignin, were positioned in strong negative association with digestibility indices and proximate components such as crude protein and dry matter. Morphological attributes like shoot

Table 4. Chemical composition of *Pennisetum purpureum* cv. Mott under different gamma irradiation treatments

Treatments	Nutrient composition					
	DM%	OM (%DM)	CF (%DM)	CP (%DM)	EE (%DM)	TDN (%DM)
Un irradiated	88.29±0.30[a]	86.28±0.60	31.62±1.34	10.45±0.86	4.59±0.44	60.59±1.24
IR 5	88.74±0.34[a]	86.57±0.86	31.08±1.12	9.98±0.65	4.10±0.68	60.26±1.31
IR 10	88.02±0.33[ab]	86.87±0.66	31.77±1.07	10.05±0.77	4.53±0.22	60.49±2.08
IR 15	88.09±0.54[ab]	86.65±0.73	31.54±0.68	10.12±0.72	4.32±0.58	62.75±1.33
IR 20	87.81±0.27[b]	86.78±0.43	32.67±0.22	9.31±0.58	4.08±0.55	59.92±1.73
IR 25	88.27±0.41[ab]	86.91±0.89	32.17±0.50	10.79±0.79	4.17±0.25	61.20±1.24
IR 30	87.79±0.24[b]	87.23±0.63	32.76±0.51	10.02±0.71	4.42±0.17	60.59±1.58
p	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Note: Data are presented as mean ± standard deviation. Different superscript letters within the same column indicate significant differences at p<0.05 according to Duncan's Multiple Range Test (DMRT). Treatments included unirradiated control (0 Gy) and gamma irradiation doses of 5, 10, 15, 20, 25, and 30 Gy. DM: dry matter; OM: organic matter; CF: crude fiber; CP: crude protein; EE: ether extract; TDN: total digestible nutrients. All nutrient compositions are expressed on a dry matter basis (%DM).

Table 5. Fiber fraction content of *Pennisetum purpureum* cv. Mott under different gamma irradiation treatments

Treatments	Fiber fraction contents					
	NDF%	ADF%	Cellulose%	Hemicellulose%	ADL	Lignin%
Un irradiated	67.56±2.97[bc]	35.84±1.57	31.73±1.40[a]	34.06±1.60[a]	2.50±0.22[a]	1.78±0.12[a]
IR 5	66.73±2.63[abc]	36.25±1.66	30.48±1.21[a]	33.85±1.85[ab]	3.27±0.41[ab]	2.40±0.48[ab]
IR 10	71.51±4.17[a]	37.22±0.73	35.55±1.64[ab]	35.28±0.24[a]	3.03±1.20[ab]	2.18±0.69[ab]
IR 15	63.98±1.76[c]	33.10±1.68	30.89±1.29[a]	31.02±1.67[cd]	2.31±0.37[a]	2.07±0.12[ab]
IR 20	71.08±2.60[a]	36.68±2.19	34.40±0.59[b]	32.63±0.24[bc]	3.77±0.92[b]	2.81±0.41[bc]
IR 25	69.77±2.47[ab]	35.07±1.57	34.70±1.84[b]	31.92±1.39[cd]	3.83±0.91[b]	3.15±1.04[c]
IR 30	70.49±4.22[ab]	35.83±2.00	34.66±2.29[b]	30.53±0.19[d]	7.25±0.71[c]	6.20±0.53[d]
p	<0.05	>0.05	<0.01	<0.01	<0.01	<0.01

Note: Data are presented as mean ± standard deviation. Different superscript letters within the same column indicate significant differences at p<0.05 or p<0.01 according to Duncan's Multiple Range Test (DMRT). Treatments included unirradiated control (0 Gy) and gamma irradiation doses of 5, 10, 15, 20, 25, and 30 Gy. Measured parameters included neutral detergent fiber (NDF, %), acid detergent fiber (ADF, %), cellulose (%), hemicellulose (%), acid detergent lignin (ADL, %), and lignin (%).

Table 6. The fiber fraction digestibility of *Pennisetum purpureum* cv. Mott treated with gamma irradiation

Treatments	Nutrient digestibility						
	DMD%	OMD%	IVD (%DM)	NDFD%	ADFD%	CeD%	HmD%
Un irradiated	58.03±0.84^{ac}	58.18±0.51^{ac}	68.23±0.88^{acd}	54.06±1.56^{a}	52.81±2.00^{ab}	54.71±2.18^{ab}	55.48±1.76^{ab}
IR 5	57.93±1.79^{ac}	57.83±1.66^{ac}	65.82±2.99^{cd}	48.98±3.40^{b}	48.87±5.76^{b}	50.35±5.46^{b}	53.47±4.51^{b}
IR 10	61.27±1.55^{b}	61.48±1.74^{b}	71.08±1.01^{ab}	59.45±2.92^{c}	56.07±2.79^{a}	57.77±3.01^{a}	59.02±1.63^{ac}
IR 15	60.23±1.50^{ab}	60.08±1.65^{ab}	71.56±2.38^{a}	53.64±2.33^{a}	53.05±2.65^{ab}	55.72±2.84^{a}	54.14±2.15^{b}
IR 20	60.88±2.68^{ab}	60.91±2.36^{ab}	70.38±2.09^{ac}	58.35±1.71^{c}	55.82±2.25^{a}	58.26±2.33^{a}	61.02±1.79^{c}
IR 25	55.59±2.41^{c}	55.33±2.58^{c}	66.05±1.71^{d}	51.26±3.69^{ab}	48.28±4.19^{b}	49.58±3.49^{b}	54.26±3.27^{b}
IR 30	56.88±2.18^{c}	56.89±2.35^{c}	66.54±2.68bcd	53.84±3.20^{a}	51.19±3.79^{ab}	50.19±3.84^{b}	56.57±2.79^{ab}
p	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01

Note: Data are presented as mean ± standard deviation. Different superscript letters within the same column indicate significant differences at p<0.05 or p<0.01 according to Duncan's Multiple Range Test (DMRT). Treatments included unirradiated control (0 Gy) and gamma irradiation doses of 5, 10, 15, 20, 25, and 30 Gy. Measured parameters included dry matter digestibility (DMD, %), organic matter digestibility (OMD, %), *in vitro* digestibility (IVD, %DM), neutral detergent fiber digestibility (NDFD, %), acid detergent fiber digestibility (ADFD, %), cellulose digestibility (CeD, %), and hemicellulose digestibility (HmD, %).

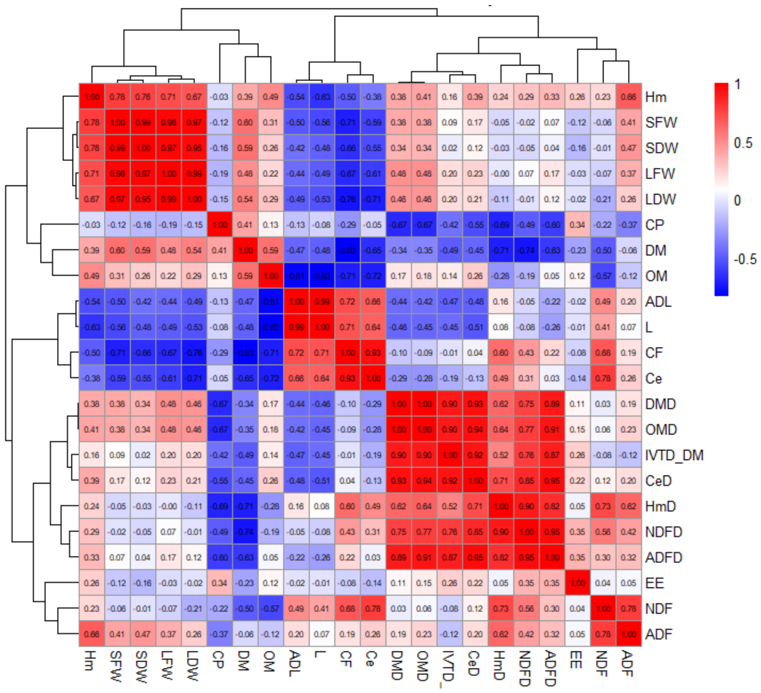

Figure 2. Hierarchical correlation analysis of gamma radiation effects on morphological and nutritional traits in *Pennisetum purpureum* cv. Mott. Abbreviations used in the figure include: Hm = hemicellulose, SFW = stem fresh weight, SDW = stem dry weight, LFW = leaf fresh weight, LDW = leaf dry weight, CP = crude protein, DM = dry matter, OM = organic matter, ADL = acid detergent lignin, L = lignin, CF = crude fiber, Ce = cellulose, DMD = dry matter digestibility, OMD = organic matter digestibility, IVD_DM = *in vitro* digestibility of dry matter, CeD = digestible s, HmD = digestible hemicellulose, NDFD = digestible neutral detergent fiber, ADFD = digestible acid detergent fiber, EE = ether extract, NDF = neutral detergent fiber, and ADF = acid detergent fiber.

fresh weight, shoot dry weight, and leaf biomass were grouped with yield-related variables, showing weaker or inverse correlations with fiber digestibility. Overall, the heatmap distinguished two broad clusters: one centered on productivity and proximate composition, and the other driven by digestibility and fiber degradation dynamics.

Principal Component Analysis (PCA)

The PCA results (Figures 3–4) demonstrated that the first two components captured a substantial portion of the total variation, providing clear discrimination among irradiation treatments. Plants exposed to lower doses (5–10 Gy) separated distinctly from both the unirradiated control and the high-dose groups, highlighting the dose-dependent shifts in trait expression. Variables such as leaf number, stem diameter, leaf and stem biomass, and IVD were strongly loaded on the first principal component, contrasting with lignin-related traits that projected in the opposite direction.

The control group occupied a more neutral position in the biplot, overlapping partially with the 15–20 Gy treatments. Variable contribution analysis (Figure 5) indicated that digestibility traits (DMD, OMD, IVD, and NDFD), together with cell wall characteristics, accounted for most of the variance across treatments.

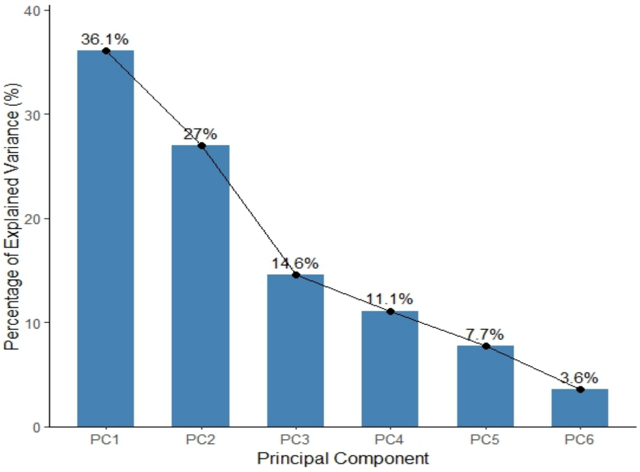

Figure 3. Scree plot from principal component analysis (PCA) showing the percentage of trait variance explained by each principal component in *Pennisetum purpureum* cv. Mott.

Morphological traits contributed at an intermediate level, while TDN, stem diameter, and ether extract showed minimal influence on treatment separation. Collectively, the PCA highlighted digestibility and structural carbohydrate traits as the dominant factors underlying the multivariate response to gamma irradiation.

DISCUSSION

Gamma irradiation has emerged as a potent tool for inducing genetic variability in forage crops, with the ability to alter morphological, physiological, and biochemical traits (Raina *et al*., 2022). In this study, the application of gamma rays on *Pennisetum purpureum* cv. Mott produced a spectrum of variations that were functionally relevant to both plant productivity and forage quality. The mutants derived from low to moderate doses (particularly 10–20 Gy) exhibited

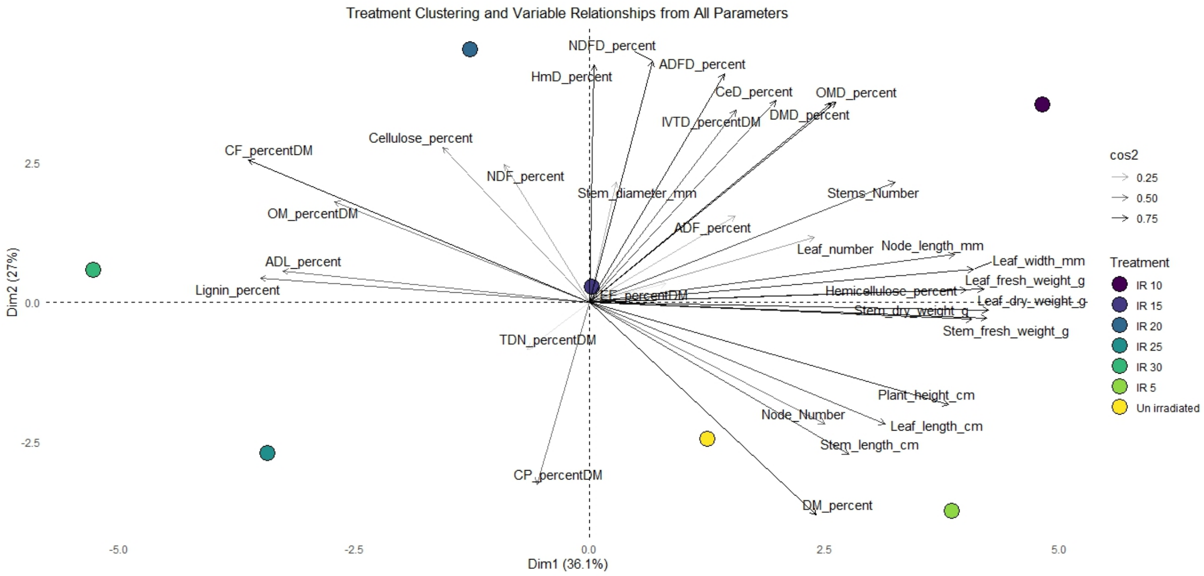

Figure 4. Principal component analysis biplot of gamma irradiation effects on *Pennisetum purpureum* cv. Mott traits.

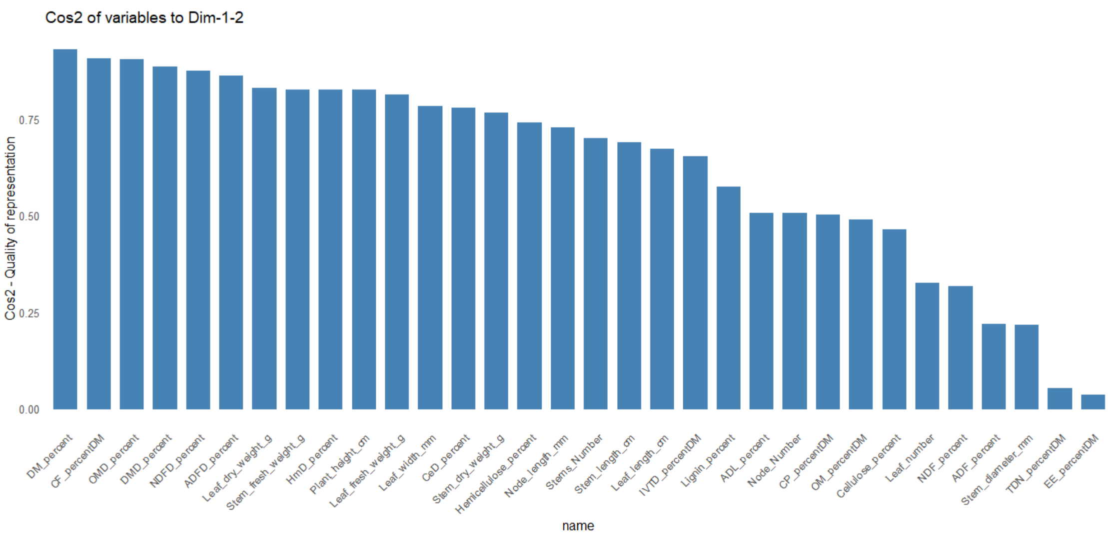

Figure 5. Cos² values indicating variable representation on principal component analysis (PCA) dimensions 1 and 2. This bar plot illustrates the *cos²* (squared cosine) values of all analysed variables, reflecting their quality of representation on the first two principal components (PC1 and PC2) derived from the PCA.

marked improvements in plant height, number of tillers, leaf area, and both fresh and dry biomass yields. The positive response observed at moderate doses is consistent with the principle of hormesis, in which sublethal stress can stimulate physiological functions rather than suppress them (Cutler *et al*., 2022). At the cellular scale, low-dose irradiation often triggers a transient oxidative burst that activates antioxidant defense pathways, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Rao *et al*., 2025). Strengthening these defense mechanisms enables plants to neutralize reactive oxygen species (ROS) more effectively and sustain cellular homeostasis, ultimately supporting improved metabolism and growth (Dumanović *et al*., 2021).

Gamma irradiation also promotes meristematic activity and modulates hormonal regulation, particularly through auxin, cytokinin, and gibberellin signaling, which govern cell division and elongation (Jan *et al*., 2012). As a result, plants exposed to moderate radiation exhibit greater vigor and higher biomass accumulation. This hormetic stimulation is reinforced by antioxidant protection that reduces oxidative damage and maintains metabolic efficiency (Franco *et al*., 2019). However, once the dose exceeds approximately 15 Gy, these advantages diminish. High irradiation levels compromise DNA integrity, lead to excessive ROS buildup, induce lipid peroxidation, and disrupt protein synthesis, collectively impairing growth. Under such stress, the repair systems are overwhelmed, hormonal balance is disturbed, and meristematic tissues are damaged, producing shorter internodes, fewer leaves, reduced cell division, and lower biomass yield (Wahab *et al*., 2022).

In addition to these morphological responses, gamma irradiation influenced nutritional traits. A notable effect was the increase in crude protein (CP) at moderate doses, which may be associated with improved nitrogen assimilation and shifts in metabolic flux toward amino acid and protein production. This outcome could involve the activation of key enzymes in nitrogen metabolism, such as nitrate reductase and glutamine synthetase, enabling more efficient conversion of inorganic nitrogen into organic compounds required for protein synthesis (Zayed *et al*., 2023). However, the differences in CP were not statistically significant across treatments, and other proximate components—crude fiber (CF), ether extract (EE), and ash—remained largely unchanged. This stability suggests that the core metabolic pathways regulating protein accumulation, structural fiber deposition, lipid synthesis, and mineral uptake were not markedly altered by the levels of irradiation applied. Crude fiber (CF) levels remained relatively constant among treatments, indicating that overall deposition of structural carbohydrates in the cell wall was stable, even though specific fiber components such as NDF, ADF, and lignin varied. Likewise, the absence of significant changes in ether extract (EE) suggests that lipid metabolism was largely unaffected by irradiation, while the consistent ash values point to an ability of the plants to maintain mineral acquisition and ion balance under both irradiated and non-irradiated conditions.

Shifts in fiber composition were evident at certain irradiation levels, with notable reductions in NDF, ADF, and ADL, indicating partial inhibition of lignification. Since lignin is a major barrier to forage digestibility due to its resistance to microbial degradation and its cross-linking with cellulose and hemicellulose, this decline provides clear benefits for nutrient availability in ruminants (Zhong *et al*., 2021). These changes are likely linked to gamma-induced alterations in the phenylpropanoid pathway, involving reduced activity or downregulation of key enzymes such as PAL, COMT, CAD, and related peroxidases, which collectively limit lignin monomer production and polymerization (Shad *et al*., 2024). As a result, lignin deposition in secondary cell walls decreases, while the structural arrangement of cellulose and hemicellulose is modified in ways that increase microbial access. In some cases, slight increases in cellulose or hemicellulose appeared as compensatory adjustments, but overall the outcome was greater cell wall porosity and flexibility (Wang *et al*., 2021). Taken together, these modifications improve cell wall degradability and enhance the digestible energy value of the forage.

As a result, irradiated plants demonstrated superior digestibility, with significant improvements in dry matter digestibility (DMD), organic matter digestibility (OMD), *in vitro* digestibility (IVD), and fiber digestibility (NDFD and ADFD). These enhancements point to increased availability of fermentable substrates and reduced structural barriers within the cell wall, both of which are critical for optimizing forage utilization in ruminant production systems (Tedeschi *et al*., 2023). Mechanistically, reduced lignin content together with altered cellulose and hemicellulose composition enlarges pore size and surface area of the cell wall, facilitating microbial colonization and enzymatic hydrolysis (Weimer, 2022). This improved accessibility leads to faster nutrient release and absorption, which can translate into higher feed intake and better animal performance (Reuben *et al*., 2022). Such outcomes underscore the functional integration between plant biochemical composition and its nutritional performance under digestive simulation.

The multivariate analysis also highlighted a crucial aspect: while biomass-related traits (e.g., shoot weight, leaf length, and dry weight) were crucial for yield, they did not always align positively with digestibility traits. This finding reinforces the importance of holistic breeding strategies that account for both yield and quality traits, rather than focusing on biomass alone (Khan *et al*., 2024). This suggests underlying trade-offs in metabolic allocation between structural growth and forage quality. Plants operate under finite resource allocation; thus, resources invested heavily into structural components for increased biomass (e.g., extensive lignification for mechanical support) might divert precursors and energy from processes that contribute to higher nutritional value or digestibility. This dynamic interplay between growth and defense/quality mechanisms is a key physiological response to environmental and genetic perturbations (Katam *et al*., 2022). The clustering dendrogram (Figure 2) further underscored this by segregating variables into distinct groups: one for morphological productivity traits and proximate contents, and another for digestibility traits and cell wall degradability indicators.

A particularly compelling aspect of this research lies in the environmental context—namely, the cultivation of plants on post-mining land, specifically former gold mine sites. These soils are typically characterized by poor soil fertility, heavy metal contamination, and damaged soil structure, including high acidity, low nutrient status, and low organic matter (Zine *et al*., 2024). These conditions represent a highly stressful environment that limits plant establishment and growth (Putra *et al*., 2022b). That the gamma-irradiated mutants not only survived but outperformed controls under such marginal conditions (as shown by their advantageous positions in the biplot, Figure 4), indicates their improved adaptability (Riviello-Flores *et al*., 2022). This likely stems from enhanced root system architecture, allowing for greater nutrient acquisition efficiency in nutrient-poor soils, and possibly upregulation of antioxidant systems and phytochelatin production for metal detoxification (Ali *et al*., 2024).

Phytochelatins, small peptides, and metallothioneins, proteins, function by chelating heavy metal ions in the cytoplasm, sequestering them into vacuoles, thereby reducing their toxic effects and enhancing plant tolerance to contaminated soils (Faizan *et al*., 2024). Such resilience is vital for reclaiming degraded lands through phytoremediation-compatible forage systems. Functionally, these findings bridge plant productivity with ecosystem restoration. The selected mutants, by exhibiting high biomass, superior nutritional quality, and tolerance to abiotic stress, serve as dual-purpose genotypes—contributing to livestock feed security while also supporting ecological rehabilitation of degraded landscapes. The use of gamma irradiation has therefore shown promise not only in enhancing desirable agronomic traits but also in generating adaptive traits that permit successful cultivation in harsh and contaminated environments.

CONCLUSION

Gamma irradiation at moderate doses (10–15 Gy) effectively improved the growth, nutritional quality, and digestibility of *Pennisetum purpureum* cv. Mott. Mutants derived from these treatments performed well even on gold mine tailing soils, indicating strong adaptability. These findings demonstrate the potential of mutation breeding as a sustainable strategy for developing superior forage cultivars while contributing to land rehabilitation and livestock production.

CONFLICT OF INTEREST

L. Abdullah serves as editor of the Tropical Animal Science Journal but has no role in the decision to publish this article. The authors also declare that there is no conflict of interest. All procedures and interpretations were conducted independently and without any commercial or financial relationships that could be construed as a potential conflict of interest.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this manuscript, the author(s) used *Grammarly* to assist in checking grammar, spelling, and language clarity. After using this tool, the author(s) carefully reviewed and edited the content as needed and take full responsibility for the final content of the publication.

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