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Morphological and Molecular Diversity of Five Superior Napier Grass Cultivars in Indonesia

M. D. M. Nasution[a], N. Umami[b,*], A. Kurniawati[b], & M. M. Rahman[c]
[a]Doctoral Student in Animal Science, Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia
[b]Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia
[c]Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan 90509, Sabah, Malaysia
*Corresponding author: nafiatul.umami@ugm.ac.id
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ABSTRACT

This study aimed to evaluate the morphological and genetic diversity among five cultivars of Napier grass (*Pennisetum purpureum*) grown in Indonesia: 'Gama Umami', 'Pakchong', 'Odot', 'Purple', and 'Local'. A total of 20 plants per cultivar were planted in a completely randomized design with morphological parameters, nutrient content, biomass production, and molecular analysis assessed at a cutting age of 90 days. The morphological analysis revealed significant differences ($p<0.05$) in plant height, leaf length, and the number of tillers across the cultivars. Qualitative analysis revealed differences in leaf color and growth habits. 'Gama Umami' cultivar showed the highest biomass yield, with significantly ($p<0.05$) higher crude protein and dry matter content than the other cultivars. Random amplified polymorphic DNA (RAPD) analysis using nine primers on the five Napier grass cultivars demonstrated diverse band patterns, resulting in a percentage of polymorphic bands (PBP) ranging from 60% to 100%. The dendrogram derived from the RAPD data clustered the cultivars into two main groups, with 'Gama Umami' and 'Local' showing a high similarity coefficient of 0.73, while 'Purple' and 'Pakchong' formed a distinct sub-cluster with a similarity coefficient of 0.66, and 'Odot' exhibited a similarity coefficient of 0.58 with the 'Purple' and 'Pakchong' sub-cluster. This study revealed significant genetic and morphological diversity among five Napier grass cultivars, with 'Gama Umami' demonstrating superior morphological traits, nutrient content, and biomass production. These findings highlight the potential of integrating molecular and morphological analyses to support breeding programs for improving forage quality and productivity.

***Keywords:** cultivar; molecular; morphology; Pennisetum purpureum; randomly amplified polymorphic DNA*

INTRODUCTION

Napier grass, also known as elephant grass (*Pennisetum purpureum* Schum), is a tetraploid plant (2n = 4x = 28) originating from sub-Saharan Africa (Muktar *et al.*, 2023). This plant is commonly used as livestock feed in tropical and subtropical regions (Umami *et al.*, 2022) due to its high biomass production, good nutritional content, and versatility in various applications such as silage (Mudhita *et al.*, 2024) and hay (Kamruzali *et al.*, 2021). It has been introduced and established in over 100 tropical and subtropical countries worldwide, representing more than 50% of the global Napier grass population. Additionally, approximately 80% of the forage consumed by cattle in many tropical and subtropical countries comes from Napier grass (Islam *et al.*, 2023). Despite its widespread use, there is a significant lack of comprehensive information regarding the genetic diversity and agronomic potential of various Napier grass cultivars in Indonesia. This knowledge gap hinders effective breeding programs and the selection of superior cultivars.

The number of Napier grass cultivars is huge due to its open pollination system and wide genetic diversity resulting from natural crossbreeding. *P. purpureum* includes about 140 cultivars, with more than 300 accessions available in various gene banks worldwide (Negawo *et al.*, 2017). Over time, new genotypes continue to develop by introducing and breeding the existing cultivars.

Napier grass has been introduced throughout tropical regions and grows naturally in Southeast Asia. The introduction of Napier grass to Indonesia began with the arrival of the Hawaii and Africa cultivars, followed by various cultivars such as Taiwan and Mott, which are the result of genetic breeding (Mansyur *et al.*, 2019). In Indonesia, extensive exploration and research have been conducted on numerous Napier grass cultivars, including 'Gama Umami', 'Biovitas', 'Pakchong', 'Zanzibar', 'Tifton', 'King Thailand', 'Taiwan', 'Wrukwona', 'Odot', and 'Local' (Ernawati *et al.*, 2023; Umami *et al.*, 2022).

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These cultivars exhibit various advantages, reflecting improvements in agronomic performance.

The identification of several Napier grass cultivars is crucial for germplasm information due to the lack of information on the existing cultivars. Several studies have highlighted the potential of these cultivars, yet comprehensive genetic characterization is lacking. Therefore, cultivar identification greatly supports and continues genetic breeding or plant improvement (Swarup *et al.,* 2021). Additionally, it can provide information on the presence or absence of intrinsic variability in each cultivar (de Lima *et al.,* 2011). Characterization and evaluation of cultivars can be conducted through morphological and molecular information. However, molecular markers have the advantage of revealing genetic differences in more detail without being influenced by environmental factors (Hasan *et al.,* 2021). Nevertheless, morphological identification combined with genetic analysis can aid in data interpretation and the formation of more accurate heterotic groups.

Genetic diversity studies on plant cultivars, including Napier grass, often utilize molecular markers such as Random Amplified Polymorphic DNA (RAPD) (Figueiredo Daher *et al.,* 2002; Passos *et al.,* 2005; Babu *et al.,* 2009; de Lima *et al.,* 2011). This study will utilize RAPD markers to provide a detailed genetic assessment of the selected cultivars, which has not been extensively explored in previous research on Napier grass in Indonesia. This technique effectively detects genetic polymorphism and distinguishes individuals with morphological similarities across various cultivars. The RAPD is considered ideal for diversity evaluation due to its highly polymorphic nature, providing information on genetic variation and relationships among cultivars (Korir *et al.,* 2013). Studies on genetic diversity using RAPD in Indonesia have also been conducted on plants such as *Saccharum officinarum* (Hapsoro *et al.,* 2015). *Oryza sativa* (Zakiyah *et al.,* 2019), and *Glycine max* (Wahyudi *et al.,* 2020).

This study aimed to examine the morphological and genetic diversity among five Napier grass cultivars ('Gama Umami', 'Pakchong', 'Odot', 'Purple', and 'Local') in Indonesia using RAPD markers. By elucidating the genetic diversity within these cultivars, this research seeks to contribute to the improved decision-making in future plant breeding efforts, ultimately enhancing the productivity and quality of forage resources in Indonesia.

MATERIALS AND METHODS

Study Site Condition

Field research was conducted over three months from December 2023 to March 2024 at the experimental field of the Forage and Pasture Laboratory, Faculty of Animal Science, Universitas Gadjah Mada (latitude: -7.769238, longitude: 110.386085), Indonesia. The climate conditions at the research location were recorded by the Meteorology, Climatology, and Geophysics Agency in Sleman, Yogyakarta, during the study period (Figure 1). The soil at the location was classified as regosol. Before the study began, soil samples were taken at a 0 to 20 cm depth from 10 sampling points and combined for chemical analysis (Table 1).

Plant Materials and Experimental Design

Five Napier grass cultivars, consisting of 'Gama Umami', 'Pakchong', 'Odot', 'Purple', and 'Local', were used in this study (Figure 2). The experimental field was prepared by plowing and dividing it into 20 plots before planting, covering an area of 150 m². Each row consisted of 5 plots, with a total of 5 rows, resulting in 25 plots. Each plot measured 1 m × 1 m, with a 1 m distance between plots. A total of 4 plants of the same cultivar were planted in each plot, resulting in 20 plants per cultivar. The placement of the plants within the plots was randomized using a block random design to ensure a fair representation of each cultivar. Planting was done using cuttings laid horizontally. Daily watering was performed in the morning and evening, and weeding was done weekly. Defoliation was performed 90 days after planting. This study included morphological observations, nutrient content evaluation, biomass production measurement, and molecular analysis using Random Amplified Polymorphic DNA (RAPD) techniques. Data collected were analyzed to assess the diversity among the cultivars and provide insights for future breeding programs.

Morphology Observations

Morphological measurements and observations were conducted on 20 individual plants for each cultivar. These measurements and observations were recorded 90 days after planting. Morphological

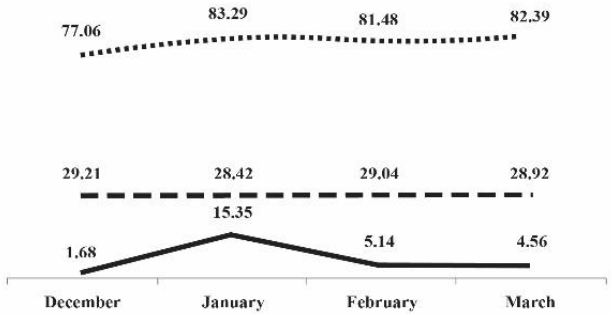

Figure 1. Temperature (- -, °C), humidity (●●, %), and rainfall (—, mm) conditions at the research site based on data from the Meteorology, Climatology, and Geophysics Agency (BMKG) Sleman, Yogyakarta in 2024.

Table 1. Soil analysis at the experimental site in the field of the Forage and Pasture Laboratory, Faculty of Animal Science, Universitas Gadjah Mada

Parameters	Value	Criteria (score)[b]
pH (H_2O)	7.05	Neutral soil (6.6-7.3)
C-Organic (%)	3.25	Medium (3-8.75)
Organic matter (%)	5.61	High (5.16-15)
N-Total (%)	0.25	Medium (0.15-0.25)

Note: [b] Hazelton & Murphy (2016).

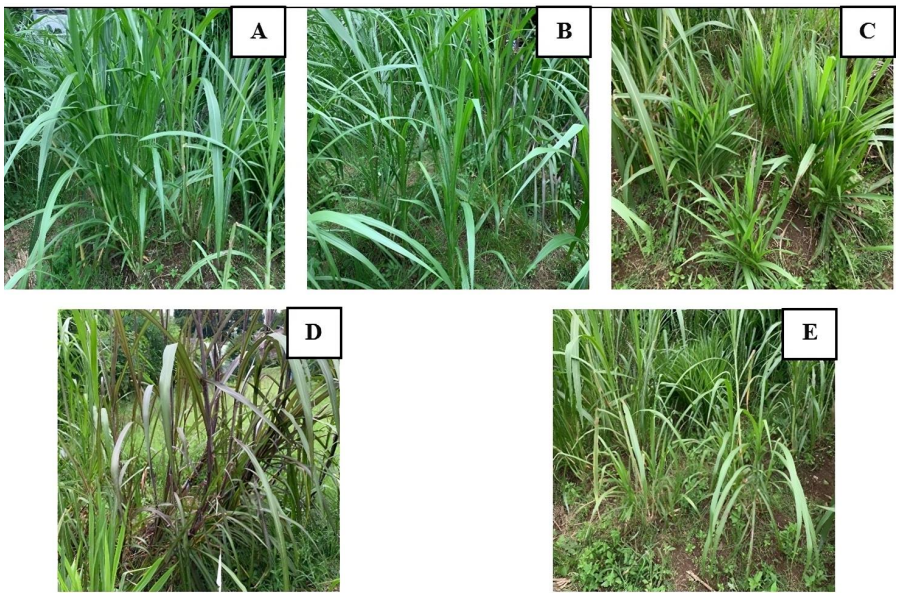

Figure 2. Five Napier grass (*Pennisetum purpureum*) cultivars used in the study. Cultivar Gama Umami (A), Pakchong (B), Odot (C), Purple (D), and Local (E).

measurements included plant height (cm), plant length (cm), stem diameter (cm), leaf length (cm), leaf width (cm), number of leaves, number of tillers, and number of nodes. Other morphological data were also characterized using parameters based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 4246 of 2019 regarding forage plant varieties and the UPOV (International Union for the Protection of New Varieties of Plants) descriptor list (UPOV, 2010).

Evaluation of Nutrient Contents and Biomass Production

Nutrient content and biomass production evaluations of the five Napier grass cultivars were conducted to compare the cultivars and their potential as forage plants. The assessments were made on plants harvested at 90 days old. Samples from the entire plant, harvested 10 cm above the ground, were chopped into small pieces using a chopper machine. The samples were then packed in newspaper and immediately dried at 55 °C for 72 hours. After drying, the samples were ground using a Wiley mill to pass through a 1 mm sieve for proximate analysis.

The chemical composition of the samples was analyzed according to AOAC (2005) standards for dry matter (DM), organic matter (OM), crude fiber (CF), and crude protein (CP), while ether extract (EE) was measured using the method by Kamal (1997). Extract material without nitrogen (EMWN) and total digestible nutrients (TDN) were calculated based on chemical analysis results using formulas from Hartadi *et al.* (2005). Crude protein was determined through nitrogen (N) concentration analysis using the Kjeldahl method and calculated by multiplying the N content by 6.25 (CP= N × 6.25). Total DM, OM, and CP production were calculated by multiplying biomass production by the chemical composition (DM, OM, CP) (%) of fresh forage production.

DNA Extraction

Samples from five cultivars were prepared by extracting and purifying 0.05 g of fresh young leaves. Genomic DNA was isolated from the fresh young lower leaves of the plants using the cetyl trimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990), modified with the addition of 1% mercaptoethanol. The isolated DNA template was subjected to quantitative and qualitative testing to determine DNA concentration and purity. Quantitative testing was performed by measuring absorbance at 260:280 nm using a NanoDrop 1000. Qualitative testing was conducted using electrophoresis on 0.8% (w/v) agarose gel, then viewed on a UV transilluminator. The obtained DNA was then used as a template for PCR reactions.

RAPD-PCR Analysis

At the beginning of the experiment, 11 RAPD primers were selected for all cultivar samples to detect variability and suitability in Napier grass cultivars (Table 2). Primers were chosen based on references from previous RAPD studies. The primers used were selected based on the consistency of the bands produced. The RAPD amplification was then performed with genomic DNA extracted and purified from the five Napier grass cultivars. The PCR reactions were carried out with a total volume of 10 µL for each PCR tube. Each PCR reaction consisted of 5 µL Go Taq® Green Master Mix (Promega, USA), 0.25 µL 100 µM primer (Sigma-Proligo), 2.5 µL DNA sample, and 2.25 µL nuclease-free water.

The DNA amplification was performed using a BOECO PCR System. The amplification reaction included denaturation at 94 °C for 30 seconds, followed by primer annealing at 37 °C for 30 seconds, and elongation at 72 °C for 2 minutes, with a final elongation at 72 °C for 10 minutes. The PCR products were then

Table 2. RAPD primer profile used and the level of polymorphism detected for 5 Napier grass (*Pennisetum purpureum*) cultivars with different RAPD primers

RAPD primer	5′ – 3′ sequence	Primers chosen	TNB	NMB	NPB	PBP (%)	SR (bp)
OPA 1	CAGGCCCTTC	Not chosen	14	14	0	0	400 - 2000
OPA 6	CGTCCCTGAC	Not chosen	0	0	0	0	-
OPA 9	GGGTAACGCC	Chosen	9	0	9	100	280 - 1600
OPB 5	TGCGCCCTTC	Not chosen	0	0	0	0	-
OPC 11	AAAGCTGCGG	Chosen	4	0	4	100	450 - 1400
OPD 6	ACCTGAACGG	Not chosen	0	0	0	0	-
OPD 8	GTGTGCCCCA	Chosen	5	2	3	60	420 - 930
OPD 13	GGGGTGAGA	Chosen	12	0	12	100	320 - 3000
OPD 20	ACCCGGTCAC	Chosen	7	0	7	100	450 - 1500
Total			51	16	35		

Note: RAPD= Random amplified polymorphic DNA, TNB= Total number of RAPD bands, NMB= Number of monomorphic bands, NPB= Number of polymorphic bands, PBP= Polymorphic bands percentage, SR= Size ranges.

electrophoresed using 1.0% (w/v) agarose gel stained with Florosafe DNA stain in TBE buffer (consisting of 0.45 M Tris-HCl pH 8, 0.45 M boric acid, 20 mM EDTA) at 100 volts for 45 minutes. The amplification results were visualized under a UV transilluminator and photographed. A 100 bp DNA ladder (Promega, USA) was used as a standard marker. The bands were scored as 1 for presence and 0 for absence.

Data Analysis

Morphological observation data were analyzed both qualitatively and quantitatively. Qualitative data were presented descriptively, while quantitative morphological data, nutrient content, and biomass production results were statistically evaluated using one-way analysis of variance (ANOVA). The experimental design used was a Completely Randomized Design with plant cultivars as treatment. Differences between groups were then tested with Duncan's New Multiple Range Test (DMRT). Statistical analysis was performed using SPSS version 26.0 (Steel *et al*., 1997). Cluster analysis was also applied to both qualitative and quantitative morphological data using MSVP software based on Gower's similarity coefficient and the UPGMA method.

Molecular data analysis used selected primers that produced bands and showed polymorphism. The DNA from each of the five cultivars was amplified. The amplified bands were scored as 1 or 0 based on the presence or absence of bands to generate a binary data matrix. Bands found in some cultivars and absent in the others were considered "polymorphic bands," while bands present in all cultivars were considered "monomorphic bands." The resulting binary data matrix was used to calculate genetic similarity coefficients to describe intra- and inter-cultivar genetic similarities among all genotypes using NTSYS-pc software (Numerical Taxonomy System version 2.1) (Rohlf, 2000). The results from the RAPD analysis were then combined to form a single data matrix, which was used to calculate genetic distances between cultivars, estimate polymorphic bands and genetic diversity, and create a UPGMA dendrogram based on Jaccard's coefficient.

RESULTS

Morphological Characterisation

Based on the Decree of the Minister of Agriculture of the Republic of Indonesia No. 4246 of 2019 standards and UPOV descriptors, the descriptive analysis showed morphological variations among cultivars (Table 3). The 'Gama Umami' and 'Pakchong' had an upright stem habit and tall plant length, unlike 'Odot', which had a semi-upright habit and short plant length. The 'Purple' cultivar was characterized by its dominant purple leaf color, while the other cultivars had green leaves ranging from medium to dark green. Leaf length varied, with 'Gama Umami', 'Pakchong', and 'Purple' having long leaves, while 'Odot' and 'Local' had medium-length leaves. The leaf width in 'Gama Umami' and 'Pakchong' was wider than in 'Purple' and 'Local', which had a medium leaf width. Additionally, the 'Purple' cultivar was distinct due to the presence of anthocyanin, as indicated by the coloration of the nodes and internodes, which was not found in the other cultivars. Morphological assessment through measurements showed significant ($p<0.05$) variations in various parameters (Table 4). The 'Gama Umami' exhibited the greatest plant length and height, followed by 'Pakchong' and 'Purple' cultivars, while 'Odot' cultivar recorded the lowest values for both parameters. Statistical tests indicated that the differences in plant length and height among cultivars were significant ($p<0.05$). Leaf length also showed significant ($p<0.05$) variation among cultivars, with 'Gama Umami' having the longest leaf compared to 'Odot' and 'Local' cultivars. Additionally, the number of leaves in 'Gama Umami' cultivar was the highest, significantly ($p<0.05$) different from the other cultivars. The number of nodes also varied significantly ($p<0.05$), with 'Gama Umami' and 'Pakchong' showing higher number of nodes than 'Odot' and 'Local' cultivars. The number of tillers differed significantly ($p<0.05$) among cultivars, with 'Gama Umami' and 'Pakchong' exhibiting higher tiller numbers than the other cultivars.

The morphological analysis of five Napier grass cultivars is shown in Figure 3 and the similarity

matrix is shown in Figure 4. It revealed significant similarity levels among the five cultivars analyzed. The dendrogram (Figure 3) grouped the cultivars based on morphological similarities, with 'Local' and 'Purple' showing the highest similarity coefficient of 0.870, as displayed in the similarity matrix (Figure 4). Conversely, 'Gama Umami' and 'Purple' showed the lowest similarity level with a coefficient of 0.565, also reflected by the greater distance in the dendrogram. The 'Gama Umami' and 'Pakchong' formed a relatively close group with a similarity coefficient of 0.812.

Nutrient Content

The nutrient content of Napier grass varied among five cultivars (Table 5). The 'Gama Umami' cultivar had the highest DM content, significantly ($p<0.05$) different from the other cultivars, especially the 'Local' cultivar, which had the lowest DM content. The 'Odot' cultivar recorded the highest OM content, and it was significantly ($p<0.05$) different from the other cultivars, with 'Gama Umami' having the lowest OM value. The highest CF and CP contents were found in the 'Gama Umami' cultivar, and it was significantly ($p<0.05$) different from Pakchong, which had the lowest CF and CP values. The highest EE content was also found in 'Gama Umami', and it was significantly ($p<0.05$) different from the 'Purple' cultivar, which had the lowest EE value. For EMWN, the 'Odot' cultivar showed the highest value, and it was significantly ($p<0.05$) different from the 'Gama Umami' cultivar, which recorded the lowest value. Finally, the highest TDN was found in the 'Purple' cultivar, significantly ($p<0.05$) different from the 'Gama Umami' cultivar, which recorded the lowest value.

Table 3. Morphological variables of five different Napier grass (*Pennisetum purpureum*) cultivars based on the list of Decree of the Minister of Agriculture of the Republic of Indonesia No. 4246 of 2019 combined with UPOV

Variables	Cultivars				
	GU	PC	OD	PU	LK
Culm: attitude of tillers	Erect	Erect	Semi Erect	Erect	Erect
Plant: length	Tall	Tall	Short	Medium	Medium
Leaf blade: length	Long	Long	Medium	Long	Long
Width	Broad	Broad	Broad	Medium	Medium
Colour	Medium green-*Moderate yellow-green (5GY 5/6)	Dark green-*Strong yellow-green (5GY 5/8)	Dark green-*Strong yellow-green (5GY 6/8)	Purple- *Purple (7.5YR 4/4)	Medium green-*Moderate yellow-green (5GY 5/6)
Pubescence	Present	Present	Present	Present	Present
Leaf shape	Flat pointed a tip	Flat pointed a tip	Flat pointed a tip	Flat pointed a tip	Flat pointed a tip
Leaf edge texture	Denticulate	Denticulate	Denticulate	Denticulate	Denticulate
Auricle	Present	Present	Present	Present	Present
Ligule	Present	Present	Present	Present	Present
Culm: pubescence of node	Present	Present	Present	Present	Present
Diameter	Medium	Medium	Medium	Medium	Medium
Anthocyanin coloration of node	Absent	Absent	Absent	Strong	Absent
Anthocyanin coloration of internode	Absent	Absent	Absent	Strong	Absent
Shape	Silinder (stalk)	Silinder (stalk)	Silinder (stalk)	Silinder (stalk)	Silinder (stalk)
Roots: type	Fibrous	Fibrous	Fibrous	Fibrous	Fibrous
Resistance: disease	Absent	Absent	Absent	Absent	Absent

Note: *Colour charting using Munsell colour charts for plants. GU= Gama Umami, PC= Pakchong, OD= Odot, PU= Purple, LK= Local, UPOV= International Union for the Protection of New Varieties of Plants.

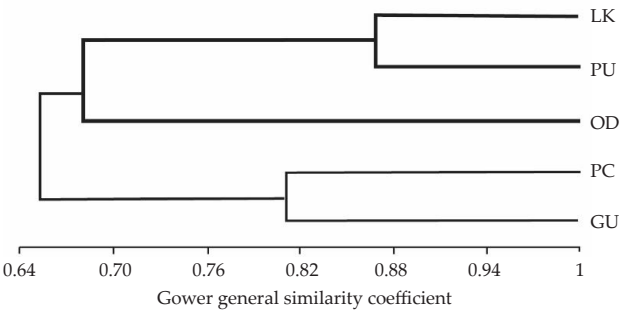

Figure 3. Dendrogram of five Napier grass (*Pennisetum purpureum*) cultivars, produced with UPGMA based on morphology data. PU= Purple, PC= Pakchong, OD= Odot, GU= Gama Umami, LK= Local.

	GU	PC	OD	PU	LK
GU	1.000				
PC	0.812	1.000			
OD	0.601	0.746	1.000		
PU	0.565	0.638	0.616	1.000	
LK	0.609	0.768	0.746	0.870	1.000

Figure 4. Similarity matrix of five Napier grass (*Pennisetum purpureum*) cultivars based on morphology. PU= Purple, PC= Pakchong, OD= Odot, GU= Gama Umami, LK= Local.

Biomass Production

The biomass yield of five Napier grass cultivars is shown in Table 6. The 'Gama Umami' cultivar had significantly ($p<0.05$) higher fresh yield than 'Odot' and 'Local' cultivars, but it was not significantly ($p>0.05$) different from 'Pakchong' and 'Purple cultivars. For DM yield, the 'Gama Umami' cultivar had a significantly ($p<0.05$) higher yield than all other cultivars, followed by 'Pakchong', which was significantly different from 'Odot' and 'Local'. The 'Gama Umami' had significantly ($p<0.05$) higher OM and CP production than the other cultivars.

Molecular Analysis using RAPD Markers

The RAPD analysis using nine primers on five Napier grass cultivars revealed diverse band patterns, with five selected primers showing significant polymorphism, as shown in Table 2 and Figure 5. Primers OPA 9, OPC 11, OPD 8, OPD 13, and OPD 20 produced different polymorphic bands, with a percentage of band polymorphism (PBP) ranging from 60% to 100%. The bands produced by these primers showed size variations from 280 bp to 3000 bp, indicating genetic diversity among the five cultivars.

The dendrogram generated from the RAPD data (Figure 6) grouped these five cultivars into two main clusters, with similarity coefficients ranging from 0.43 to 0.73. 'Gama Umami' and 'Local' were grouped into one sub-cluster with the highest similarity coefficient of 0.73, indicating a closer genetic relationship. 'Purple' and 'Pakchong' cultivars were also grouped into one sub-cluster with a similarity coefficient of 0.66. 'Odot' cultivar had a similarity coefficient of 0.58 with the 'Purple' and 'Pakchong' sub-cluster.

The genetic similarity matrix generated from the RAPD analysis (Figure 7) supported the dendrogram results, where 'Gama Umami' and 'Local' cultivars had the highest similarity coefficient of 0.7347. Conversely, the lowest similarity coefficient was found between 'Odot' and 'Local' cultivars, with a value of 0.1818,

Table 4. Morphological characteristics of five Napier grass (*Pennisetum purpureum*) cultivars

Variables	Cultivars				
	GU	PC	OD	PU	LK
Plant length (cm)	264 ± 16.49^{a}	239 ± 20.50^{b}	100 ± 6.09^{d}	201 ± 39.03^{c}	204 ± 15,25^{c}
Plant height (cm)	261 ± 19.27^{a}	234 ± 17.54^{b}	97 ± 5.53^{d}	214 ± 44.6^{bc}	194 ± 20.64^{c}
Number of leaves	99 ± 18.16^{a}	67 ± 5.92^{b}	77 ± 10.56^{b}	68 ± 17.64^{b}	69 ± 7.76^{b}
Leaf length (cm)	115 ± 2.11^{a}	109 ± 3.32^{b}	70 ± 1.76^{c}	109 ± 5.3^{b}	106 ± 7.1^{b}
Leaf width (cm)	4.6 ± 2.45^{a}	4.5 ± 2.79^{a}	4.1 ± 2.85^{b}	3.7 ± 1.69^{c}	3.9 ± 3.13^{b}
Stem diameter (mm)	29 ± 3.12^{a}	23 ± 1.41^{b}	25 ± 2.45^{b}	25 ± 4.22^{b}	23 ± 1.76^{b}
Number of nodes	12 ± 2.27^{a}	9 ± 0.87^{bc}	7 ± 1.22^{c}	10 ± 2.67^{a}	8 ± 1.41^{c}
Number of tillers	3 ± 0.96^{ab}	3 ± 1.63^{bc}	4 ± 1.19^{a}	2 ± 0.73^{c}	2 ± 1.37^{c}

Note: ^{abcd}Means with the different superscripts in the same row differ significantly ($p<0.05$), GU= Gama Umami, PC= Pakchong, OD= Odot, PU= Purple, LK= Local.

Table 5. Nutrient content of five Napier grass (*Pennisetum purpureum*) cultivars

Nutrient content (%)	Cultivars				
	GU	PC	OD	PU	LK
DM	19.69 ± 0.06^{a}	18.73 ± 0.06^{b}	15.68 ± 0.06^{c}	16.11 ± 0.12^{d}	15.36 ± 0.07^{e}
OM	80.43 ± 0.13^{a}	84.07 ± 0.35^{b}	86.32 ± 0.23^{c}	85.52 ± 1.17^{c}	83.71 ± 1.26^{b}
CF	33.67 ± 1.64^{a}	29.63 ± 0.09^{b}	29.84 ± 0.15^{b}	31.41 ± 0.02^{b}	30.58 ± 0.25^{b}
EE	3.10 ± 0.60^{a}	1.86 ± 0.32^{b}	2.41 ± 0.17^{ab}	1.02 ± 0.11^{c}	1.73 ± 0.66^{bc}
CP	10.92 ± 0.22^{a}	8.61 ± 0.31^{c}	9.95 ± 0.24^{b}	9.37 ± 0.19^{b}	9.53 ± 0.15^{b}
EMWN	34.48 ± 1.60^{a}	45.32 ± 0.37^{b}	45.50 ± 0.37^{b}	44.91 ± 1.78^{b}	43.78 ± 0.72^{b}
TDN	43.73 ± 0.53^{a}	51.82 ± 0.29^{b}	53.27 ± 0.06^{b}	56.30 ± 0.98^{b}	54.49 ± 3.55^{b}

Note: ^{abcd} Means with the different superscripts in the same row differ significantly ($p<0.05$), DM= Dry matter, OM= Organic matter, CF= Crude fiber, EE= Ether extract, CP= Crude protein, EMWN= Extract material without nitrogen, TDN= Total digestible nutrient, GU= Gama Umami, PC= Pakchong, OD= Odot, PU= Purple, LK= Local.

Table 6. Biomass yield of five Napier grass (*Pennisetum purpureum*) cultivars

Production (tons/ha)	Cultivars				
	GU	PC	OD	PU	LK
Fresh	27.33 ± 5.03^{a}	20.00 ± 7.54^{ab}	13.33 ± 3.78^{b}	18.33 ± 2.51^{ab}	13.93 ± 3.05^{b}
DM	6.10 ± 0.52^{a}	3.74 ± 1.41^{b}	2.09 ± 0.59^{c}	2.95 ± 0.38^{bc}	2.04 ± 0.46^{c}
OM	24.93 ± 2.10^{a}	16.80 ± 6.28^{b}	11.50 ± 3.24^{b}	15.69 ± 2.33^{b}	11.13 ± 2.41^{b}
CP	3.54 ± 0.01^{a}	1.43 ± 0.60^{b}	1.54 ± 0.02^{b}	1.74 ± 0.36^{b}	1.14 ± 0.25^{b}

Note: ^{abcd} Means with the different superscripts in the same row differ significantly ($p<0.05$), DM= Dry matter, OM= Organic matter, CP= Crude protein, GU= Gama Umami, PC= Pakchong, OD= Odot, PU= Purple, LK= Local.

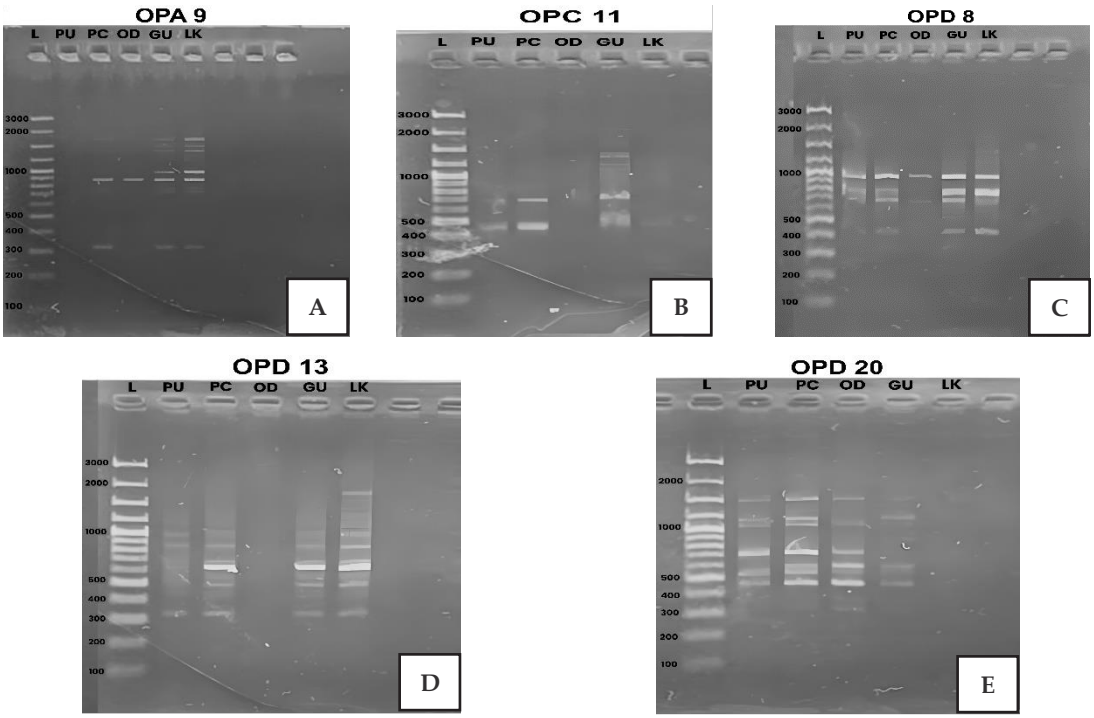

Figure 5. Random amplified polymorphic DNA bands of five Napier grass (*Pennisetum purpureum*) cultivars using chosen primers. OPA 9 (A), OPC 11 (B), OPD 8 (C), OPD 13 (D), OPD 20 (E). L= Marker DNA ladder, PU= Purple, PC= Pakchong, OD= Odot, GU= Gama Umami, LK= Local.

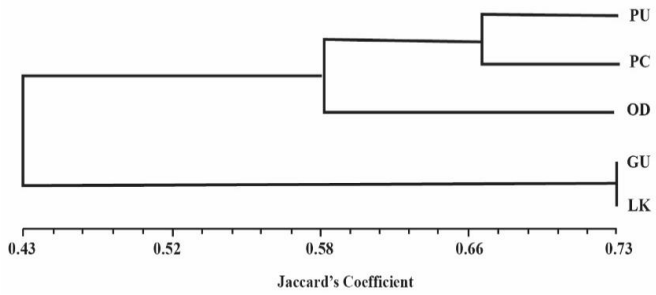

Figure 6. Dendrogram of five Napier grass (*Pennisetum purpureum*) cultivars, produced with UPGMA based on random amplified polymorphic DNA data. PU= Purple, PC= Pakchong, OD= Odot, GU= Gama Umami, LK= Local.

	GU	PC	OD	PU	LK
GU	1.000				
PC	0.708	1.000			
OD	0.279	0.500	1.000		
PU	0.450	0.667	0.667	1.000	
LK	0.735	0.578	0.182	0.378	1.000

Figure 7. Genetic similarity matrix of five Napier grass (*Pennisetum purpureum*) cultivars based on Random Amplified Polymorphic DNA. PU= Purple, PC= Pakchong, OD= Odot, GU= Gama Umami, LK= Local.

indicating the most significant genetic difference among the five cultivars.

DISCUSSION

Morphological Diversity

The analysis of morphological characteristics of the five Napier grass cultivars showed significant variations in both qualitative and quantitative aspects. Qualitative factors, such as leaf color, plant growth type, and the presence of anthocyanin, provide a clear basis for distinguishing one cultivar from another. The 'Purple' cultivar had purple leaves due to its high anthocyanin content, unlike the other cultivars like 'Gama Umami' and 'Local', which had yellowish-green leaves. According to Onjai-uea *et al.* (2023), the 'Purple' cultivar contained anthocyanins that ranged from red to purple in its leaves and stems, with total anthocyanin content ranging from 0.57 mg/g to 2.52 mg/g. Differences in plant growth type are also evident in 'Odot', which has a shorter size and semi-erect habit, reflecting its dwarf nature not found in the other cultivars.

Additionally, quantitative data further supported this analysis, where the 'Gama Umami' cultivar consistently showed the highest performance in quantitative morphological variables. Conversely, 'Odot' cultivar recorded the lowest values in most quantitative variables, aligning with its qualitative characteristics as a dwarf cultivar. According to Rocha *et al.* (2017), the 'Odot' cultivar showed a lower average plant height than the other cultivars due to its dwarf gene. These findings are consistent with Animasaun *et al.* (2017), who stated that vegetative traits in individual plants are correlated, showing significant relationships between morphological characteristics.

Differences in plant height and length among cultivars can also result from the combination of genetic variability and environmental influences. These

differences indicate genetic variation among the cultivars and highlight their varying adaptation potentials. Furthermore, the morphological variations found reflect the outcomes of the breeding processes conducted. This statement also aligns with the findings of Respati *et al.* (2018), who reported that environmental factors and the genetic traits of the plants can influence differences in plant morphology and size.

This analysis is further supported by dendrogram data generated from the UPGMA method (Figure 3) and the similarity matrix (Figure 4). The dendrogram showed clear groupings among cultivars, with 'Local' and 'Purple' having the highest similarity coefficient of 0.870, indicating a very close morphological relationship. Conversely, the 'Gama Umami' and 'Purple' cultivars showed the lowest similarity level with a coefficient of 0.565, indicating significant differences in morphological characteristics between these cultivars. These results demonstrated that although some traits had similarities or overlaps, fundamental genetic differences can still be detected through morphological analysis and supported by dendrogram grouping.

According to McBenedict *et al.* (2016), some morphological traits that show similarities are due to genetic characteristics expressed at various levels. This finding indicated that certain traits can be strongly, moderately, or weakly expressed in the same genotype. Animasaun *et al.* (2017) reported that intraspecific variation in vegetative parameters in cultivars was due to differences in genetic composition or internal genetic control of accessions.

Overall, the combination of qualitative and quantitative morphological data with the results from the dendrogram and similarity index provides an overview of the genetic diversity among Napier grass cultivars. These insights have important implications for breeding programs, where a deeper understanding of the genetic relationships among cultivars can be used to develop new, more adaptive, and productive varieties. These results also indicated the potential for utilizing specific cultivars in specific environmental conditions, which can enhance the efficiency of forage and biomass production.

Nutrient Content

The obtained nutrient content showed significant variation, indicating different genetic potentials among the cultivars. The 'Gama Umami' cultivar provided the highest results in several parameters, such as DM and CP contents. These findings indicated great potential in agronomic applications to improve livestock feed quality. The high DM content in 'Gama Umami' offers storage durability and production efficiency advantages. The DM content obtained in this study was influenced by genetic factors and the other factors, such as plant age and environmental conditions. The DM content of elephant grass ranged from 18% to 28%, with an average of 20% to 22%. These differences might be influenced by several factors, such as seasons, plant age, soil fertility, and cultivation management (Tilahun *et al.*, 2017; Jaime *et al.*, 2019).

Higher CP content indicated its ability to support more efficient livestock growth. The significant genetic variation among these cultivars plays an important role in determining the capacity of cultivars to assimilate nutrients from the soil, which ultimately reflects in the quality of the nutrients produced. Delevatti *et al.* (2019) stated that the protein content in forage is greatly influenced by the availability of nitrogen contributed by the soil.

Variation among cultivars leads to differences in morphological characteristics, where plants with higher growth rates tend to have higher nutritional quality (Cid *et al.*, 2008). Greater vegetative growth allows cultivars to absorb more nutrients from the soil, which are then accumulated in plant tissues, enhancing nutrient content. The genetic diversity among these cultivars affects not only nutrient content but also the plants' ability to adapt to various environmental conditions, affecting energy use efficiency. These results also indicated that selecting the right cultivar can maximize the nutritional potential of feed and energy efficiency, ultimately supporting overall livestock productivity.

Biomass Production

Biomass production is beneficial in breeding programs and in evaluating the nutritional value of forage, helping in the selection and differentiation of different cultivars, and can contribute to the characterization of elephant grass biomass for energy use (Ampong-Nyarko & Murray, 2011).

High biomass production in Napier grass is influenced by leaf size and number, stem size, number of nodes, and tillers, where greater leaf length, width, and plant height increase photosynthetic capacity and optimize vegetative growth (Rodrigues *et al.*, 2014; Animasaun *et al.*, 2018; Calzada-Marín *et al.*, 2024; Pinchi-Carbajal *et al.*, 2024). These findings are consistent with the results of this study, where the 'Gama Umami' cultivar had the highest biomass production among the cultivars, followed by high results in morphological parameters.

Molecular Diversity using RAPD Markers

The selected primers were chosen because they produced clear, unambiguous, reproducible, and polymorphic DNA bands. The bands amplified by the primers provided genetic information, allowing cultivars to be differentiated. A total of 37 bands were detected based on the five polymorphic primers. According to Babu *et al.* (2009), genomic DNA amplification from 30 *P. purpureum* genotypes produced 7 to 14 bands per primer used. In contrast, de Lima *et al.* (2011) produced 2 to 8 bands per primer used.

The bands produced and the high polymorphism is crucial for accurately estimating the genetic diversity of germplasm collections (Azevedo *et al.*, 2012). Primers that produce few bands are unable to differentiate the DNA samples of the cultivars used. The percentage of polymorphic bands for the RAPD markers produced in this study was quite high, as shown in Table 2. Previous

studies on Napier grass germplasm analyzed through RAPD markers reported polymorphism levels of 77% (Passos *et al.,* 2015), 56.2% (Daher *et al.,* 2002), and 86.6% (Babu *et al.,* 2009).

Differences in band patterns observed in the RAPD molecular analysis results of Napier grass cultivars, aside from the success of primers in identifying them, can also be attributed to genetic changes such as mutations, deletions, or homologous recombination. These changes can affect the binding sites of oligonucleotide primers, resulting in new bands not detected in the other cultivars. Similar phenomena have been reported in soybean plants, where changes in oligonucleotide priming sites due to mutations and homologous recombination led to the appearance of new bands (Dhakshanamoorthy *et al.,* 2015).

The dendrogram results based on UPGMA analysis, shown in Figure 6, produced two major groups with similarity coefficients ranging from 0.43 to 0.73. Group one consisted of the 'Purple', 'Pakchong', and 'Odot' cultivars, while group two consisted of the 'Gama Umami' and 'Local' cultivars. These groupings indicated that these genotypes were genetically different. The grouping results and the highest similarity values suggested that the cultivars may have the same genetic ancestors (Azevedo *et al.,* 2012; Hapsoro *et al.,* 2015; Rocha *et al.,* 2017). According to Babu *et al.* (2009), 30 Napier grass accessions were classified into three main clusters originating from the same location, with similarity coefficients ranging from 0.63 to 0.93.

Based on the similarity index results shown in Figure 7, the lower the similarity index value between cultivars, the better their breeding potential. This relationship exists because greater genetic distance indicates more significant genetic differences. In this study, the low similarity index between 'Odot' and 'Local' (0.182) and 'Gama Umami' and 'Odot' (0.279) indicated substantial genetic differences, making them potential candidates for breeding strategies to increase genetic diversity. The high variation in the Napier grass population is due to its tetraploid nature, high heterozygosity, and the broad diversity of its parents (Azevedo *et al.,* 2012).

Additionally, the similarity index results in Figure 7 indicated that the origin of the plants did not influence the genetic distance or similarity between cultivars. This finding is supported by Hapsoro *et al.* (2015), who stated that although genotypes originate from the same geographical region, they can be genetically different. Furthermore, the results indicated that genotypes from various geographical regions can be genetically similar. Several factors influence genetic structure, including breeding systems, genetic drift, population age and size, environmental heterogeneity, seed dispersal, gene flow, evolutionary history, and natural selection (Wanjala *et al.,* 2013).

RAPD markers effectively detected diversity among cultivars in this study. The advantages of these markers include their use for anonymous genomes and low DNA amounts, as well as producing a high number of DNA fragments, effectively identifying individual variability in populations and plant breeding (Arif *et al.,* 2010; Kumari & Thakur, 2014; Yongjun *et al.,* 2014; Wahyudi *et al.,* 2020). Breeding or genetic improvement programs, in addition to selecting plants with high agronomic traits, also consider those with distant relationships (González & Martínez, 2019). The genetic distance coefficient used for breeding materials varies, with values between 0.50 and 0.47 recommended for parents (Kandel *et al.,* 2016). However, Azevedo *et al.* (2012) suggested cultivars with a minimum genetic distance of 0.85.

Correlation between Diversity of Morphological and Molecular

The number of polymorphic bands produced by several RAPD primers demonstrated the effectiveness of these markers in distinguishing each genotype. However, these primers could not group cultivars with similar morphological characteristics, as molecular analysis reveals genetic variations that are not always apparent in morphology, which is also influenced by environmental factors. According to Negawo *et al.* (2017), integrating phenotypic and genotypic data to support molecular breeding in Napier grass is still limited. Therefore, combining morphological and molecular analyses is crucial for grouping closely related cultivars. Mansyur *et al.* (2019) added that further observations related to morphology, production, and nutrient content are needed to evaluate the potential of cultivars as new forage varieties.

The importance of combining morphological analysis with biomass production and nutrient content is to provide a comprehensive understanding of genetic diversity and phenotypic expression and its impact on agronomic quality. Molecular markers alone are insufficient to group cultivars, as the relationship between genetics and phenotype is often complex (Rocha *et al.,* 2017). Similarly, relying solely on morphology is ineffective because morphology is influenced by environmental and plant management factors (Kandel *et al.,* 2016). Low genetic similarity between parents with high economic value in morphology, nutrient content, and biomass production is important for the conservation and management of germplasm. Combining morphological, molecular, and agronomic analyses provides a more accurate assessment of genetic diversity and cultivar potential. This integrated approach helps identify superior varieties for breeding programs and conserves valuable genetic resources.

CONCLUSION

This study revealed significant diversity among five Napier grass cultivars, with 'Gama Umami' showing superior morphological traits, the highest crude protein and dry matter content, and the greatest biomass yield, supporting its potential as a high-quality forage cultivar. The molecular analysis using RAPD also revealed genetic diversity among the cultivars, which had the potential to support further breeding programs. The integration of morphological, nutrient, and genetic analyses is crucial for the development of superior varieties. These findings highlight the importance of

a comprehensive approach in breeding programs to enhance genetic diversity and improve cultivar performance.

CONFLICT OF INTEREST

We certify that there are no conflicts of interest, financial, personal, or otherwise, with any individuals or organizations related to the material discussed in this manuscript.

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REFERENCES

Ampong-Nyarko, K. & Murray, C. (2011). Utility of forage grass nutrient composition databases in predicting ethanol production potential. Journal of Biobased Materials and Bioenergy, 5, 295–305. https://doi.org/10.1166/jbmb.2011.1167

Animasaun, D. A., Rathod, H. P., & Krishnamurthy, R. (2018). Analysis of forage yield and nutritional contents of *Pennisetum glaucum* (pearl millet) and *Pennisetum purpureum* (Napier grass) accessions. Cuban Journal of Agricultural Science, 52(4), 447-455.

Animasaun, D., Morakinyo, J., Krishnamurthy, R., & Mustapha, O. (2017). Genetic divergence of Nigerian and Indian pearl millet accessions based on agronomical and morphological traits. Journal of Agricultural Sciences, Belgrade, 62(2), 115–131. https://doi.org/10.2298/JAS1702115A

AOAC. (2005). Official methods of analysis (16th ed.). Association of Official Analytical Chemists, International.

Arif, I. A., Bakir, M. A., Khan, H. A., Al Farhan, A. H., Al Homaidan, A. A., Bahkali, A. H., Al Sadoon, M., & Shobrak, M. (2010). Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. Genetics and Molecular Research, 9(4), 2191–2198. https://doi.org/10.4238/vol9-4gmr848

Azevedo, A. L. S., Costa, P. P., Machado, J. C., Machado, M. A., Van der Pereira, A., & da Silva Lédo, F. J. (2012). Cross species amplification of *Pennisetum glaucum* microsatellite markers in *Pennisetum purpureum* and genetic diversity of Napier grass accessions. Crop Science, 52(4), 1776–1785. https://doi.org/10.2135/cropsci2011.09.0480

Babu, C., Sundaramoorthi, J., Vijayakumar, G., & Ram, S. G. (2009). Analysis of genetic diversity in Napier grass (*Pennisetum purpureum* Schum) as detected by RAPD and ISSR markers. Journal of Plant Biochemistry and Biotechnology, 18(2), 181–187. https://doi.org/10.1007/BF03263317

Calzada-Marín, J. M., Ortega-Jiménez, E., Enríquez-Quiroz, J. F., Vaquera-Huerta, H., Escalante-Estrada, J. A. S., & Antonio-Medina, A. (2024). Analysis of the growth of Chetumal grass established in a tropical climate. Agro Productividad, 16(12), 147-153. https://doi.org/10.32854/agrop.v16i12.2781

Cid, M. S., Ferri, C. M., Brizuela, M. A., & Sala, O. (2008). Structural heterogeneity and productivity of a tall fescue pasture grazed rotationally by cattle at four stocking densities. Japanese Society of Grassland Science, 54(1), 9–16. https://doi.org/10.1111/j.1744-697X.2008.00099.x

de Lima, R. S. N., Daher, R. F., Gonçalves, L. S. A., Rossi, D. A., do Amaral Júnior, A. T., Pereira, M. G., & Lédo, F. J. S. (2011). RAPD and ISSR markers in the evaluation of genetic divergence among accessions of elephant grass. Genetics and Molecular Research, 10(3), 1304–1313. https://doi.org/10.4238/vol10-3gmr1107

Delevatti, L. M., Cardoso, A. S., Barbero, R. P., Leite, R. G., Romanzini, E. P., Ruggieri, A. C., & Reis, R. A. (2019). Effect of nitrogen application rate on yield, forage quality, and animal performance in a tropical pasture. Scientific Reports, 9(1), 7596. https://doi.org/10.1038/s41598-019-44138-x

Dhakshanamoorthy, D., Selvaraj, R., & Chidambaram, A. (2015). Utility of RAPD marker for genetic diversity analysis in gamma rays and ethyl methane sulphonate (EMS)-treated Jatropha curcas plants. Comptes Rendus - Biologies, 338(2), 75–82. https://doi.org/10.1016/j.crvi.2014.12.002

Doyle, J. J. & Doyle, J. J. (1990). Isolation of plant DNA from fresh tissue. Focus, 12(1), 13–15.

Ernawati, A., Abdullah, L., Permana, I. G., & Karti, P. D. M. H. (2023). Forage production and nutrient content of different elephant grass varieties cultivated with *Indigofera zollingeriana* in an intercropping system. Tropical Animal Science Journal, 46(3), 321–329. https://doi.org/10.5398/tasj.2023.46.3.321

Figueiredo Daher, R., Gonzaga Pereira, M., Vander Pereira, A., & Teixeira do Amaral Jr, A. (2002). Genetic divergence among elephant grass cultivars assessed by RAPD markers in composit samples. Scientia Agricola, 59(4), 623–627. https://doi.org/10.1590/S0103-90162002000400001

González, C. & Martínez, R. O. (2019). Genetic characterization of clones and varieties of *Cenchrus purpureus* with microsatellite markers. Cuban Journal of Agricultural Science, 53(3), 307-318.

Hapsoro, D., Warganegara, H. A., Utomo, S. D., Sriyani, N., & Yusnita. (2015). Genetic diversity among sugarcane (*Saccharum officinarum* L.) genotypes as shown by randomly amplified polymorphic DNA (RAPD). Agrivita, 37(3), 247–257. https://doi.org/10.17503/Agrivita-2015-37-3-p247-257

Hartadi, H., Reksohadiprojo, S., & Tillman, A. D. (2005). Feed composition table for Indonesia: Vol. Fifth Printing. Gadjah Mada University Press.

Hasan, N., Choudhary, S., Naaz, N., Sharma, N., & Laskar, R. A. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programs. Journal of Genetic Engineering and Biotechnology, 19(1), 128. https://doi.org/10.1186/s43141-021-00231-1

Hazelton, P. & Murphy, B. (2016). Interpreting soil test results: what do all the numbers mean? (2nd ed.). CSIRO Publishing. https://doi.org/10.1071/9781486303977

Islam, M. R., Garcia, S. C., Sarker, N. R., Islam, M. A., & Clark, C. E. F. (2023). Napier grass (*Pennisetum purpureum* Schum) management strategies for dairy and meat production in the tropics and subtropics: yield and nutritive value. Frontiers in Plant Science, 14, 1269976. https://doi.org/10.3389/fpls.2023.1269976

Jaime, A., Rosemberg, M., & Echevarría, M. (2019). Effect of age and season on the yield and nutritive value of Morado elephant grass (*Pennisetum purpureum* x *Pennisetum americanum*) in the central coast. Scientia Agropecuaria, 10(1), 137–141. https://doi.org/10.17268/sci.agropecu.2019.01.15

Kamal, M. (1997). Kontrol Kualitas Pakan. Fakultas Peternakan, Universitas Gadjah Mada.

Kamruzali, M. A., Rahman, M. M., Mat, K., Rusli, N. D., &

Umami, N. (2021). Effects of cutting process and drying period using sunlight on hay quality of dwarf Napier grass (*Pennisetum purpureum*) and *Asystasia gangetica*. Pertanika Journal of Tropical Agricultural Science, 44(3), 685–695.

Kandel, R., Singh, H. P., Singh, B. P., Harris-Shultz, K. R., & Anderson, W. F. (2016). Assessment of genetic diversity in Napier grass (*Pennisetum purpureum* Schum.) using microsatellite, single-nucleotide polymorphism and insertion-deletion markers from Pearl Millet (*Pennisetum glaucum* [L.] R. Br.). Plant Molecular Biology Reporter, 34(1), 265–272. https://doi.org/10.1007/s11105-015-0918-2

Korir, N. K., Han, J., Shangguan, L., Wang, C., Kayesh, E., Zhang, Y., & Fang, J. (2013). Plant variety and cultivar identification: Advances and prospects. Critical Reviews in Biotechnology, 33(2), 111–125. https://doi.org/10.3109/07388551.2012.675314

Kumari, N. & Thakur, S. K. (2014). Randomly amplified polymorphic DNA-a brief review. American Journal of Animal and Veterinary Sciences, 9(1), 6–13. https://doi.org/10.3844/ajavsp.2014.6.13

Mansyur, Karti, P. M., Abdullah, L., Husni, A. L. I., & Lestari, P. (2019). Genetic diversity of mutant napiergrass using expressed sequence tag simple sequence repeat (EST-SSR). Biodiversitas, 20(8), 2403–2409. https://doi.org/10.13057/biodiv/d200839

McBenedict, B., Chimwamurombe, P., Kwembeya, E., & Maggs-Kölling, G. (2016). Genetic diversity of Namibian *Pennisetum glaucum* (L.) R. BR. (Pearl Millet) landraces analyzed by SSR and morphological markers. Scientific World Journal, 1439739. https://doi.org/10.1155/2016/1439739

Mudhita, I. K., Putra, R. A., Rahman, M. M., Widyobroto, B. P., Agussalim, & Umami, N. (2024). The Silage quality of *Pennisetum purpureum* cultivar Gamma Umami mixed with *Calliandra calothyrsus* and *Lactiplantibacillus plantarum*. Tropical Animal Science Journal, 47(1), 112–124. https://doi.org/10.5398/tasj.2024.47.1.112

Muktar, M. S., Bizuneh, T., Anderson, W., Assefa, Y., Negawo, A. T., Teshome, A., Habte, E., Muchugi, A., Feyissa, T., & Jones, C. S. (2023). Analysis of global Napier grass (*Cenchrus purpureus*) collections reveals high genetic diversity among genotypes with some redundancy between collections. Scientific Reports, 13(1), 14509. https://doi.org/10.1038/s41598-023-41583-7

Negawo, A. T., Teshome, A., Kumar, A., Hanson, J., & Jones, C. S. (2017). Opportunities for napier grass (*Pennisetum purpureum*) improvement using molecular genetics. Agronomy, 7(2), 28. https://doi.org/10.3390/agronomy7020028

Onjai-uea, N., Paengkoum, S., Taethaisong, N., Thongpea, S., Sinpru, B., Surakhunthod, J., Meethip, W., Purba, R. A. P., & Paengkoum, P. (2023). Effect of cultivar, plant spacing and harvesting age on yield, characteristics, chemical composition, and anthocyanin composition of purple Napier grass. Animals, 13(1), 10. https://doi.org/10.3390/ani13010010

Passos, L. P., Machado, M. A., Vidigal, M. C., & Campos, A. L. (2005). Molecular characterization of elephant grass accessions through RAPD markers. Ciência e Agrotecnologia, 29(3), 568–574. https://doi.org/10.1590/S1413-70542005000300009

Pinchi-Carbajal, S. F., Quispe-Ccasa, H. A., Ampuero-Trigoso, G., Nolasco-Lozano, E., & Saucedo-Uriarte, J. A. (2024). Morphological and productive correlations of cutting *Pennisetum* varieties under conditions of peruvian humid tropics. Tropical Animal Science Journal, 47(3), 363–370. https://doi.org/10.5398/tasj.2024.47.3.363

Respati, A. N., Umami, N., & Hanim, C. (2018). Growth and production of *Brachiaria brizantha* cv. MG5 in three different regrowth phases treated by Gamma radiation dose. Tropical Animal Science Journal, 41(3), 179–184. https://doi.org/10.5398/tasj.2018.41.3.179

Rocha, J. R. A. S. C., Machado, J. C., Carneiro, P. C. S., Carneiro, J. C., Resende, M. D. V., Lédo, F. J. S., & Carneiro, J. E. S. (2017). Bioenergetic potential and genetic diversity of elephantgrass via morpho-agronomic and biomass quality traits. Industrial Crops and Products, 95, 485–492. https://doi.org/10.1016/j.indcrop.2016.10.060

Rodrigues, R. C., Sousa, T. V. R., Melo, M. A. A., Araújo, J. S., Lana, R. P., Costa, C. S., Oliveira, M. E., Parente, M. O. M., & Sampaio, I. B. M. (2014). Agronomic, morphogenic and structural characteristics of tropical forage grasses in northeast Brazil. Tropical Grasslands-Forrajes Tropicales 2(2), 214-222. https://doi.org/10.17138/TGFT(2)214-222

Rohlf, F. J. (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system version 2.1. Exeter Publishing Setauket.

Steel, R. G. D., Torrie, J. H., & Dicky, D. A. (1997). Principles and procedures of statistics, a biometrical approach (3rd ed.). McGraw Hill, Inc. Book Co.

Swarup, S., Cargill, E. J., Crosby, K., Flagel, L., Kniskern, J., & Glenn, K. C. (2021). Genetic diversity is indispensable for plant breeding to improve crops. Crop Science, 61(2), 839–852. https://doi.org/10.1002/csc2.20377

Tilahun, G., Asmare, B., & Mekuriaw, Y. (2017). Effects of harvesting age and spacing on plant characteristics, chemical composition and yield of desho grass (*Pennisetum pedicellatum* Trin.) in the highlands of Ethiopia. Tropical Grasslands-Forrajes Tropicales, 5(2), 77–84. https://doi.org/10.17138/TGFT(5)77-84

Umami, N., Respati, A. N., Rahman, M. M., Umpuch, K., & Gondoe, T. (2022). Somatic embryogenesis and plant regeneration from the apical meristem of Wrukwona Napiergrass (*Pennisetum purpureum*) treated with thidiozuron and cupric sulfate. Tropical Animal Science Journal, 45(2), 220–226. https://doi.org/10.5398/tasj.2022.45.2.220

UPOV. (2010). Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability: TG/260/1. International Union for the Protection of New Varieties of Plants: Geneve, Switzerland. https://www.upov.int/edocs/tgdocs/en/tg260.pdf

Wahyudi, D., Hapsari, L., & Sundari. (2020). RAPD analysis for genetic variability detection of mutant soybean (*Glycine max* (L.) Merr). Journal of Tropical Biodiversity and Biotechnology, 5(1), 68–77. https://doi.org/10.22146/jtbb.53653

Wanjala, B. W., Obonyo, M., Wachira, F. N., Muchugi, A., Mulaa, M., Harvey, J., Skilton, R. A., Proud, J., & Hanson, J. (2013). Genetic diversity in Napier grass (*Pennisetum purpureum*) cultivars: implications for breeding and conservation. AoB Plants, 5, 1-10. https://doi.org/10.1093/aobpla/plt022

Yongjun, F., Wei, T., Jinhua, S., Tianjing, Z., Rui, Q., Bo, X., Cunyu, Z., Zhixiong, L., & Anna, Y. T. (2014). Application of random amplified polymorphic DNA (RAPD) markers to identify Taxus chinensis var. mairei cultivars associated with parthenogenesis. African Journal of Biotechnology, 13(24), 2385–2393. https://doi.org/10.5897/AJB2014.13646

Zakiyah, N. M., Handoyo, T., & Kim, K. M. (2019). Genetic diversity analysis of Indonesian aromatic rice varieties (*Oryza sativa* L.) using RAPD. Journal of Crop Science and Biotechnology, 22(1), 55–63. https://doi.org/10.1007/s12892-018-0271-0