Range Management and Agroforestry 47 (1) : 1-10, 2026
ISSN 0971-2070 (Print); 2249-5231 (Online)
https://doi.org/10.59515/rma.2026.v47.i1.25

Research article

Elucidating molecular and morpho-agronomic diversity of napier grass (*Pennisetum purpureum*) germplasm lines

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Received: 04th December, 2025 Accepted: 23rd February, 2026

Abstract

Napier grass (*Pennisetum purpureum* Schumach) is recognized for its high-yielding potential, ease of propagation and adaptability across various ecological conditions, making it a superior fodder crop. This study aimed to evaluate genetic diversity and morpho-agronomic performance among 34 Napier grass genotypes using molecular and phenotypic characterization. The experiment was conducted across two locations: Ludhiana and Gurdaspur during 2022 and 2023. Molecular diversity was assessed using 33 polymorphic SSR markers. A total of 91 alleles were detected with an average Polymorphic Information Content of 0.43, indicating that the markers were highly informative for diversity analysis. Simultaneously, the genotypes were evaluated for 12 morpho-agronomic traits to identify superior lines for nutritional quality and high fodder yield. GGE biplot analysis identified LN-29, LN-31, LN-13 and Red Napier grass as stable and high-performing genotypes for green fodder yield and quality-related traits. Correlation analysis revealed strong positive correlations between green fodder yield and several traits. Hierarchical clustering grouped the genotypes into 3 distinct clusters. Analysis of variance showed significant effects of genotype, environment and genotype x environment interaction for most traits. The comprehensive evaluation led to the identification of promising Napier grass genotypes with exceptional morpho-agronomic performance.

Keywords: Cluster analysis, Fodder, Genetic diversity, Napier grass, Pennisetum, Quality traits, SSR

Introduction

Napier grass (*Pennisetum purpureum* Schumach.), also known as elephant grass, is a polyploid perennial forage crop valued for its high biomass yield, rapid regrowth, wide adaptability and high nutritive quality (Bogdan, 1977). It plays a critical role in sustaining dairy and livestock production systems in tropical and subtropical regions, particularly in developing countries where year-round availability of quality fodder remains a major challenge (Vaghela *et al*., 2014). In India, Napier grass and its interspecific hybrids with pearl millet (bajra-napier hybrids) are extensively cultivated to meet the increasing demand for green fodder (Kumar *et al*., 2012).

Genetic improvement of Napier grass primarily targets enhanced biomass yield, multi-cut ability, stress tolerance and forage quality. However, breeding progress has been constrained by its complex polyploid genome, high heterozygosity and a narrow genetic base, which together limit heterosis exploitation and long-term genetic gains (Azevedo *et al*., 2012; Wanjala *et al*., 2013). This highlights the need for systematic assessment and effective utilization of genetic diversity in breeding programs.

Evaluation of morpho-agronomic traits remains a primary approach for identifying superior genotypes and understanding phenotypic variability. However, these traits are strongly influenced by environmental conditions, management practices and genotype x environment (GxE) interactions, which complicate accurate selection. Therefore, multi-environment testing combined with robust statistical tools like GGE biplot analysis is essential for identifying stable, high-performing genotypes (Yan *et al*., 2000; Kendal, 2016).

While phenotypic evaluation is indispensable, exclusive reliance on these traits can mask underlying genetic relationships. Molecular markers provide a complementary and more precise assessment of genetic diversity, independent of environmental influence. Among available markers, simple sequence repeats (SSRs) offer a reliable approach for assessing genetic diversity due to their co-dominant inheritance, high polymorphism and reproducibility. SSR-based diversity analysis has proven valuable for germplasm characterization and parent selection in Napier grass and related forage species (Wanjala *et al*., 2013; Muktar *et al*., 2022).

Despite these advances, integrated studies combining molecular diversity, morpho-agronomic evaluation and GGE biplot-based stability analysis in Napier grass remain limited, particularly under Indian agro-climatic conditions. Therefore, the present study aimed to (i) evaluate morpho-agronomic and forage quality traits across two environments; (ii) analyze genotype x environment interactions and yield stability using GGE biplot analysis; (iii) assess molecular genetic diversity among 34 napier grass genotypes using SSR markers and (iv) identify genetically diverse, high-yielding and stable genotypes for future breeding programs.

Materials and Methods

***Plant material and experimental design*:** A total of 34 napier grass genotypes were obtained from the Forage Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana. The experiments were conducted at two locations, viz, PAU, Ludhiana and PAU Regional Research Station (RRS), Gurdaspur, during the 2022 and 2023 growing seasons. The details of genotypes used in current research have been given in Table 1. The material was evaluated in a randomized block design (Panse and Sukhatme, 1985). Each genotype was planted in single rows of 3.6 m length with 60 cm inter-row spacing. The genotypes were replicated twice to obtain an error estimate. Standard agronomic practices were followed uniformly at both locations.

Table 1. List of source accessions

S. No.	Genotype	Designated as
1	Capricon	LN-1
2	M-30086	LN-2
3	MERKER	LN-3
4	A148	LN-4
5	K-56351	LN-5
6	K-56379	LN-6
7	N-3	LN-7
8	N-24-8	LN-8
9	TAIWAN	LN-9
10	K-52440	LN-10
11	MERKER Hybrid	LN-11
12	54256	LN-12
13	5380	LN-13
14	K-53802	LN-14
15	K-5519	LN-16
16	K-56347	LN-17
17	MERKER-532	LN-18
18	K-52504	LN-19
19	MAKE-MEGA	LN-20
20	K-5517	LN-21
21	A-5207	LN-22
22	N-23	LN-23
23	K-5240	LN-24
24	K-5182	LN-25
25	M-285303	LN-26
26	T-13	LN-27
27	N-22	LN-28
28	UGANDA	LN-29
29	CUBANO	LN-30
30	M-1-12	LN-31
31	Super Napier grass	-
32	Red Napier grass	-
33	PBN 346 (BNH)	-
34	PBN 342 (BNH)	-

***Phenotyping of morpho-agronomic and phenological traits*:** Twelve morpho-agronomic and quality traits were recorded including plant height (PH), leaf length (LL), leaf width (LW), stem girth (SG), number of leaves per plant (NOL/plant), number of tillers per plant (NOT/plant), green fodder yield (GFY), dry matter yield (DMY), crude protein (CP), *in-vitro* dry matter digestibility (IVDMD), acid detergent fiber (ADF) and neutral detergent fiber (NDF). Observations were recorded on three randomly selected plants per plot, except for yield traits, which were recorded on a whole-plot basis. Standard protocols were followed for biochemical estimation of quality traits.

DNA extraction and PCR* amplification*: Genomic DNA was extracted from young leaf tissues using the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thomson, 1980). A total of fifty SSR primers were screened initially and 33 primers showing clear and reproducible polymorphism were selected for further analysis. Polymerase chain reaction (PCR) was carried out in 20 μL volume. The annealing temperatures for the primers ranged from 52 to 62°C. The amplified products were resolved on a 6% polyacrylamide gel (PAGE).

***Statistical analysis for morpho-agronomic traits*:** All statistical analyses were performed using R software. Analysis of variance (ANOVA) across environments was conducted using a randomized block design (RBD). Genotype effect and GxE interaction (GGE) biplot analysis was performed following the model $Y_{ij} - \mu - \beta_j = g_{i1}e_{1j} + g_{i2}e_{2j} + \varepsilon_{ij}$, where β_j represents the environmental main effect, g_{i1}, g_{i2} and e_{1j}, e_{2j} are the principal component scores for genotypes and environments, respectively. The "which-won-where" and "ranking of genotypes" biplot views were generated using the R packages metan, gge, and GGEBiplotGUI. Principal component analysis (PCA) was carried out on standardized trait means, while hierarchical cluster analysis was performed using Euclidean distance and the unweighted Neighbor-Joining method in DARWIN version 6.0 (Perrier and Jacquemold-Collet, 2006) and the resulting dendrogram was used to interpret genetic divergence patterns (Saitou and Nei, 1987; Reddy *et al*., 2009).

***Genetic distance and cluster analysis*:** Molecular genetic variation among the genotypes was assessed using SSR marker data by scoring amplified bands as present (1) or absent (0) to generate a binary matrix. Genetic dissimilarity coefficients were calculated using the Dice similarity coefficient implemented in DARWIN software version 6.0 (Perrier and Jacquemold-Collet, 2006), where pairwise genetic distance (d_{ij}) between genotypes *i* and *j* was computed as $d_{ij} = \frac{b+c}{2a+(b+c)}$, with *a* representing the number of shared bands, *b* the number of bands present in genotype *i* and absent in genotype *j* and *c* the number of bands present in genotype *j* and absent in genotype *i*. The resulting dissimilarity matrix was subjected to cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) to construct a dendrogram illustrating genetic relationships among genotypes based on data from 33 polymorphic SSR primers. The polymorphic information content (PIC) was calculated using the formula $PIC = 1 - \sum p_{ij}^2$, where p_{ij} is the frequency of the *j*th allele of the *i*th marker.

Results and Discussion

Morpho-agronomic characterization

***Analysis of variance (ANOVA) for genotype, environment and G×E interactions of Napier grass genotypes*:** Mean sum of squares (Table 2) was significant ($p \leq 0.05$) for key morphological and yield traits, like PH, LL, SG, LW, NOL/plant, NOT/plant, GFY and DMY, as well as for in IVDMD, ADF, NDF. The presence of highly significant genotypic effects for most agronomic traits highlights the availability of exploitable genetic diversity, providing a strong basis for selection and improvement of napier grass genotypes.

Environmental effects were highly significant for traits like DMY, CP, IVDMD, ADF, NDF, ADL and GFY, indicating strong environmental modulation of biomass production and forage quality. In particular, the environment contributed substantially to variation in GFY and DMY, emphasizing the influence of agroclimatic conditions, soil properties and management practices on forage productivity. These findings align with earlier reports in Napier grass and other forage crops, where strong environmental effects were observed for yield and nutritional traits (Atumo *et al*., 2021; Kebede *et al*., 2017). GxE interaction effects were significant ($p \leq 0.05$) for traits, including PH, LL, SG, LW, NOL/plant, NOT/plant, GFY, DMY, CP, IVDMD, ADF and NDF, indicating differential performance of genotypes across environments. The presence of strong interaction implies that genotype rankings varied between environments, thereby justifying the need for multi-environment evaluation and stability analysis using GGE biplot methodology. Similar patterns of significant GxE interaction and wide yield variation have been reported in Napier grass across diverse agro-climatic regions, with DMY ranging from 10.05 to 13.06 t ha^{-1} (Negawo *et al*., 2018) and up to 16 t ha^{-1} under favorable environments (Kebede *et al*., 2017). Overall, the significant effects of genotype, environment and GxE interaction for most morpho-agronomic traits underline the complexity of biomass production and quality in Napier grass and highlight the necessity of integrated multivariate approaches to identify high-yielding, stable and nutritionally superior genotypes.

Mean performance of genotypes: Several genotypes, notably LN-29, LN-31, LN-13 and Red napier grass, consistently exhibited superior GFY across both environments, suggesting their broad adaptability and stable performance under different agro-climatic conditions. These genotypes therefore represent promising candidates for direct cultivation as well as for use as parental lines in breeding programs. Similar identification of high-yielding and stable Napier grass genotypes across environments has been reported in earlier studies (Jindal and Singh, 2020; Sheoran *et al*., 2017; Shashikanth *et al*., 2013).

Genotype LN-17 recorded the highest PH; a trait positively correlated with biomass accumulation in Napier grass and related forage species. Increased PH enhances leaf area development and photosynthetic capacity, ultimately contributing to improved fodder yield. Comparable associations between plant height and biomass production have been reported by Tessema *et al*. (2003), who attributed enhanced growth primarily to improved nutrient uptake efficiency. With respect to forage quality, genotypes LN-21 and LN-13 exhibited comparatively lower ADF and NDF contents, indicating superior nutritional quality. Reduced fiber fractions are associated with improved digestibility, voluntary intake and overall feeding value, making these genotypes particularly suitable for livestock feeding. Comparable

Table 2. Analysis of variance (ANOVA) for genotype, environment and G×E of napier grass genotypes

Source	df	PH (cm)		LL (cm)		SG (cm)		LW (cm)		NOL (per plant)		NOT (per plant)		GFY (kg/plot)		DMY (kg/plot)		CP (%)		IVDMD (%)		ADF (%)		NDF (%)	
		MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%	MSS	VE%
ENV	1	682835.7	4.1	7665.8	27.5	16.7	28.8	51.7	35.9	148030.9	6.1	24710.4	7.3	40.0*	0.1	5.6*	0.1	1.4**	2.0	0.007*	36.0	27.1*	1.5	92.4*	4.0
REP (ENV)	2	24566.8	6.4	6532.9	46.9	0.9*	3.2	15.6	21.7	825752.5	68.9	790.3*	4.3	8247.8	7.2	1130.8	62.2	3.2**	9.3	9.3*	9.5	64.3**	7.3	106.4*	9.3
GEN	33	380.3*	0.1	54.1*	6.4	0.4*	24.6	0.6*	15.8	4714.1*	6.4	86.9*	7.8	48.0*	6.9	9.6*	8.7	0.3	17.8	10.7*	18.1	14.3*	27.0	16.5*	23.9
GEN: ENV	33	307.2*	1.3	49.8*	5.9	0.3*	17.4	0.3*	7.3	4116.1*	5.6	103.0*	9.2	32.4*	47	7.5*	6.8	0.5*	27.6	5.3*	9.0	11.2*	21.1	16.0*	23.2
Error	66	349.0	3.0	55.2	13.1	0.2	26.0	0.4	18.9	4609.1	0.1	63.5	11.4	55.0	15.9	12.1	22.0	0.4	43.1	8.0	27.0	11.3	4.2	13.5	39.3
CV (%)		15.2		17.6		13.0		21.2		26.4		28.4		27.7		37.6		9.6		6.2		7.7		5.4	
Overall mean		122.4		42.1		3.6		3.0		256.9		28.0		26.7		9.2		6.9		45.3		43.4		68.0	

df: degree of freedom; CV: Coefficient of variation; ENV: Environment; GEN: Genotype; *(p <0.05); **(p <0.01)

trends of reduced fiber fractions in elite napier grass lines and other fodder crops have been reported by Liu *et al.* (2020) and Mohamed and Hassan (2010), reinforcing the importance of selecting genotypes combining high biomass with superior nutritive value.

Environmental influence was evident for several morphological traits, including LW, NOL/plant, reflecting the strong responsiveness of Napier grass to climatic and edaphic conditions. PH played a crucial role in determining overall GFY, consistent with findings reported by Jindal and Singh (2020) and Sheoran *et al.* (2017). DMY was strongly influenced by genotype and environmental factors, including plant spacing and harvest stage, corroborating the observations of Jindal and Singh (2020). CP content exhibited variation among genotypes and environments, with higher values generally recorded at earlier growth stages, which is consistent with the decline in protein concentration during plant maturation reported in forage crops. Collectively, the observed variation in yield and quality traits across genotypes and environments highlights the potential for simultaneous improvement of biomass productivity and forage nutritive value through targeted selection strategies.

Principal component analysis: Principal component analysis revealed that the first few (six for Ludhiana and five for Gurdaspur) components accounted for a substantial proportion of total variation, indicating that a limited number of traits predominantly govern phenotypic diversity. According to Kaiser's (1960) criteria, only factors with eigenvalues larger than 1.0 were taken under consideration. Traits such as GFY, DMY, PH, NOT/plant and LL contributed most strongly to total variability (Table 3). A biplot, for both locations, showing % of explained variance in various dimensions, is presented in fig 1. PCA further elucidated the relationships among traits, demonstrating that traits like GFY and DMY were closely associated. This insight can aid in streamlining the breeding process by focusing on key traits that enhance yield and quality.

GGE biplot analysis: GGE biplot analysis effectively revealed the patterns of genotype x environment interaction and facilitated the identification of superior and stable genotypes across environments (Fig. 2 .1–2.4). The first two principal components accounted for a major proportion of the total variation, indicating adequate representation of GxE interaction effects. The 'which-won-where' view identified LN-27 as the best-performing genotype for PH at the Gurdaspur location, highlighting its strong site-specific adaptability. Traits such as LL and NOT/plant exhibited high environmental sensitivity, suggesting that selection should emphasize genotypes with stable performance across environments. The 'ranking the genotypes' view indicated that LN-29,

Table 3. Contribution traits towards each PC's in napier grass at Ludhiana and Gurdaspur

Characters	Ludhiana						Gurdaspur				
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
PH (cm)	0.46	-0.16	-0.03	-0.08	0.62	0.12	0.38	0.09	-0.09	0.31	0.48
LL (cm)	-0.01	-0.40	0.70	-0.29	0.07	-0.05	-0.34	-0.10	0.43	-0.18	0.56
SG (cm)	0.51	-0.05	-0.21	0.22	-0.33	0.51	-0.22	0.77	0.22	0.04	-0.03
LW (cm)	0.20	0.02	0.73	-0.05	0.00	0.10	0.39	-0.23	0.21	0.47	0.38
NOT	-0.28	0.10	0.50	0.40	0.44	0.05	0.61	-0.12	-0.21	0.15	-0.14
NOL	-0.21	0.29	0.19	-0.64	0.17	-0.13	0.77	-0.25	-0.02	-0.11	0.17
GFY (kg/plot)	0.92	-0.05	-0.05	0.04	0.13	0.12	0.77	-0.01	0.12	0.17	0.12
DMY (kg/plot)	0.86	0.09	0.23	0.03	0.01	0.03	0.72	0.18	0.28	-0.05	-0.15
CP (%)	0.02	-0.31	0.03	-0.12	-0.82	0.07	-0.20	0.01	0.41	-0.72	0.16
IVDMD (%)	0.25	-0.78	-0.12	-0.18	0.07	-0.19	0.03	-0.13	-0.06	0.02	0.81
ADF (%)	-0.01	-0.08	-0.06	0.57	-0.06	-0.47	-0.14	0.60	-0.34	-0.29	0.46
NDF (%)	-0.18	0.78	0.08	0.10	0.09	0.08	-0.08	0.12	0.22	0.86	0.08

PH: Plant height; LL: Leaf length; LW: Leaf width; SG: Stem girth; NOL/plant: Number of leaves per plant; NOT/plant: Number of tillers per plant; GFY: Green fodder yield; DMY: Dry matter yield; CP: Crude protein; IVDMD: *In-vitro* dry matter digestibility; ADF: Acid detergent fiber; NDF: Neutral detergent fiber

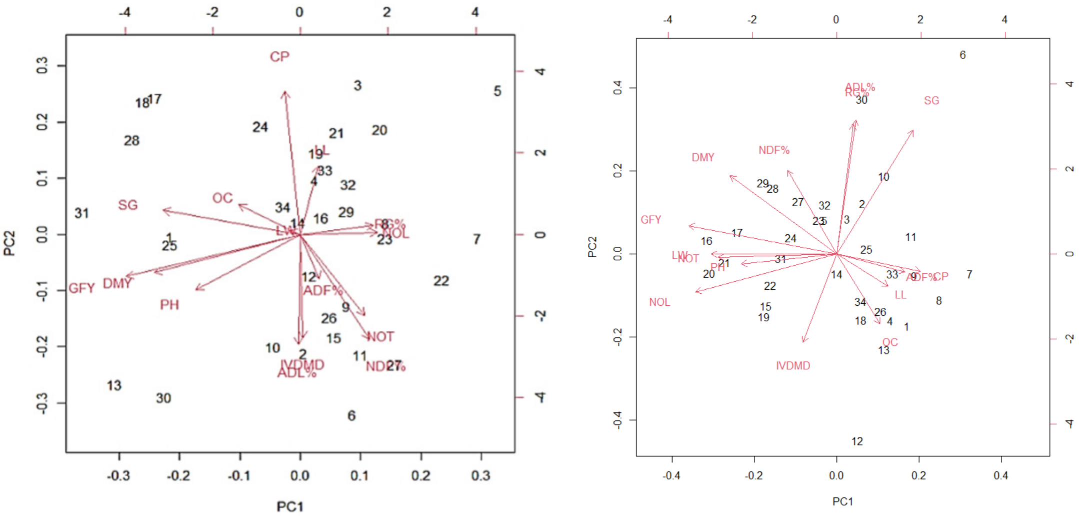

Fig 1. Biplot against PC1 and PC2 for characters (a. Ludhiana and b. Gurdaspur)

LN-31 and LN-13 combined high mean performance with superior stability for GFY, identifying them as ideal candidates for broad adaptation. Overall, the GGE biplot analysis demonstrated that effective genotype selection in Napier grass should integrate both yield potential and stability to ensure consistent performance across diverse agro-climatic conditions.

Euclidean distance and cluster analysis: The genotypes were grouped into three distinct clusters at both Ludhiana and Gurdaspur, indicating substantial genetic variability useful for breeding programs (Fig. 3 .1 and 3.2; Tables 4.1 and 4.2). At Ludhiana, the maximum inter-cluster distance (6.69) was observed between Clusters I and II, indicating high genetic divergence, while the minimum distance (5.88) occurred between Clusters II and III. Genotypes such as LN-29, LN-13, LN-31, LN-6, LN-2 and LN-16, belonging to highly divergent clusters, are promising parents for hybridization to exploit heterosis and generate superior recombinants. Cluster II exhibited the highest mean values for PH, LL, NOL/plant, NOT/plant, GFY, DMY, and CP, indicating its superiority for fodder productivity. Similar clustering trends and breeding implications were reported by Negawo *et al.* (2018). At Gurdaspur, the maximum inter-cluster distance (5.77) was observed between Clusters II

Fig 2.1. Plant height

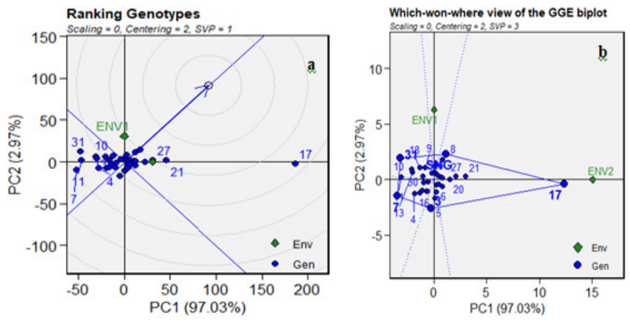

Fig 2.2. Leaf length

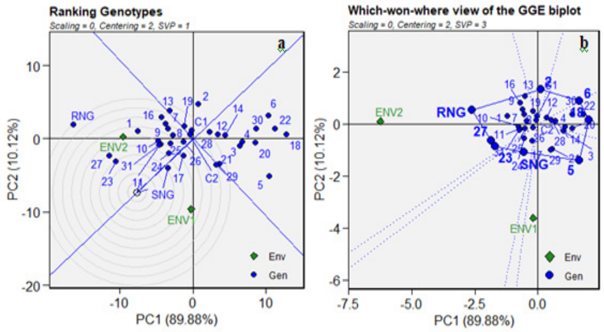

Fig 2.3. Number of tillers per plant

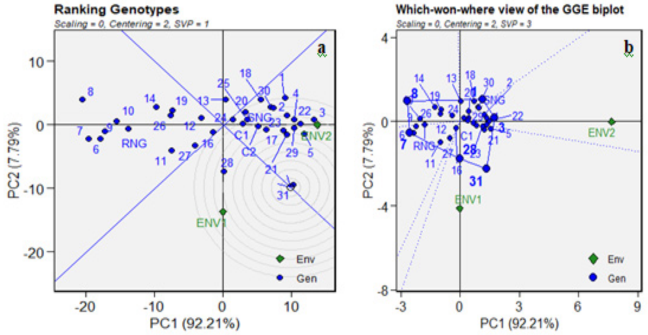

Fig 2.4. Green fodder yield

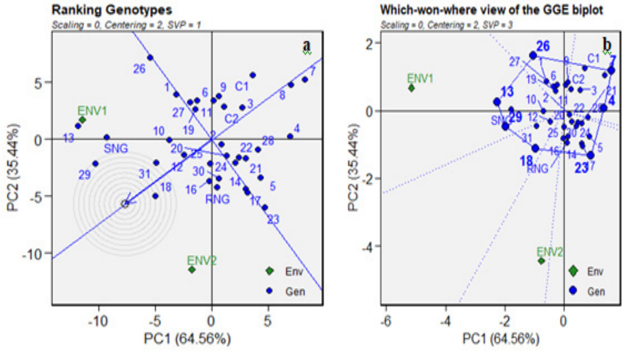

Fig 2. a) Ranking the genotypes in biplots for traits at Ludhiana and Gurdaspur; b) Which-won-where view of the GGE biplot for traits

and III, while the minimum (5.42) was between Clusters I and II. Genotypes LN-18, LN-21, LN-28, LN-29, LN-16, and LN-22 showed wide genetic divergence and are suitable candidates for future breeding programs. Cluster II recorded the highest mean values for PH, NOL/plant, NOT/plant, GFY and NDF, indicating superior biomass productivity. Overall, higher inter-cluster distances compared to intra-cluster distances confirmed substantial genetic diversity among genotypes. The identification of genetically distant, high-performing clusters provides valuable guidance for selecting parental combinations to enhance fodder yield, quality and adaptability in napier grass breeding programs (Muktar *et al*., 2022).

Correlation analysis: Correlation analysis revealed that GFY exhibited strong and significant positive associations with DMY, PH, LL and NOT/plant across both environments, indicating that these traits are the major contributors to biomass production in Napier grass (Figs. 4.1 and 4.2). DMY also showed positive correlations with PH, LL and NOT/plant, further emphasizing their critical role in determining forage productivity. Similar positive associations between GFY, PH and NOT/plant have been reported earlier in Napier grass and related forage crops (Ahmad *et al*., 2013). The strong influence of tillering capacity on sustained biomass accumulation and ratooning ability in perennial forage crops has also been highlighted by Prasojo *et al*. (2025). Comparable findings were reported by Singh *et al*. (2015), Kapoor (2021 and 2017) and Bhasker *et al*. 2017 in bajra-napier

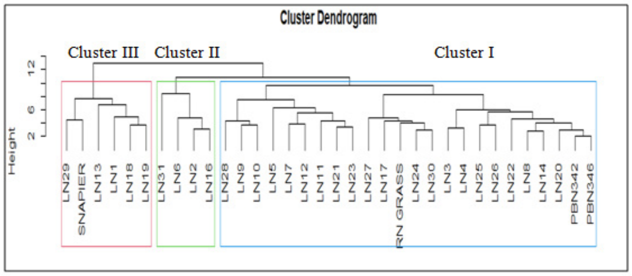

Fig 3.1. Clusters of genotypes based on fodder-related traits at Ludhiana

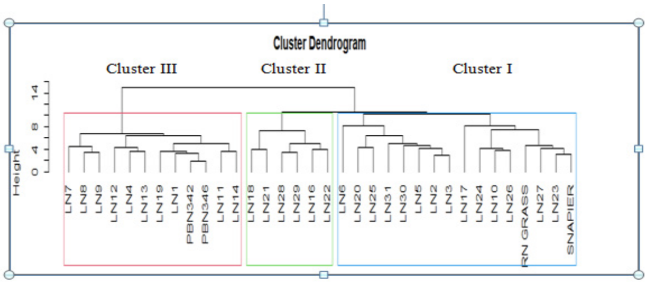

Fig 3.2. Clusters of genotypes based on fodder-related traits at Gurdaspur

Table 4.1. Average intra-cluster (diagonal) and inter-cluster distances in 34 napier grass accessions

Cluster	I	II	III
Ludhiana			
I	5.54	6.69	5.93
II		5.44	5.88
III			4.71
Gurdaspur			
I	4.22	5.42	5.77
II		5.41	5.72
III			4.70

hybrids and related fodder crops. These consistent positive correlations suggest that PH, LL and NOT/plant can serve as reliable indirect selection criteria for improving green fodder yield in Napier grass. Overall, correlation analysis highlighted significant relationships between GFY and other traits, particularly DMY and PH. The positive correlations observed suggest that selection for these traits could effectively enhance overall yield. The findings support the use of these traits as selection criteria in breeding programs aimed at improving the productivity of napier grass.

Molecular characterization

Polymorphism and allelic diversity: Out of 50 SSR primers screened, 33 (66%) exhibited clear polymorphism across the genotypes, generating a total of 91 alleles with 2 to 5 alleles per locus (Table 5). The PSMP primer series showed the highest amplification efficiency, contributing approximately 48% of the total amplified loci, indicating its high utility for genetic diversity studies in Napier grass. The PIC values varied from 0.06 (XTXP27) to 0.79 (PSMP2248), with a mean PIC of 0.43, reflecting moderate to high levels of genetic polymorphism among the evaluated genotypes. Markers with high PIC values are considered more informative and effective for discriminating closely related genotypes, suggesting that the SSR markers employed in this study were suitable for molecular characterization of polyploid forage grasses. Comparable PIC ranges 0.30 to 0.80 have been documented in Napier grass and related *Cenchrus* species, highlighting the robustness of SSR markers for genetic diversity studies (Wanjala *et al*., 2013; Muktar *et al*., 2022). The substantial allelic diversity observed in the present study aligns with Kawube *et al*. (2015), who reported 339 alleles with 27.1% unique alleles using SSR markers, indicating a broad genetic base and strong potential for parental selection in Napier grass breeding. The observed allelic richness and polymorphism in the present study likely reflect the diverse genetic background, polyploid nature and extensive breeding history of the evaluated

Table 4.2. Mean performances of different clusters evaluated for morpho-agronomic traits of 34 Napier grass accessions for fodder

Location	Clusters	PH (cm)	LL (cm)	SG (cm)	LW (cm)	NOL/ plant	NOT/ plant	GFY (kg/plot)	DMY (kg/plot)	CP (%)	IVDMD (%)	ADF (%)	NDF (%)
Ludhiana	I	125.1	41.4	3.5	2.9	255	30	26.1	8.8	6.8	44.4	44.9	68.6
	II	140.9	43.2	3.3	3.2	282	31	27.2	9.5	7.1	46.2	43.2	67.7
	III	119.1	41.6	3.4	2.4	212	22	22.2	7.7	6.9	46.0	45.8	69.28
Gurdaspur	I	201.8	50.0	4.1	2.32	223	50	27.5	9.9	7.2	43.8	45.3	67.7
	II	227.1	46.5	3.3	2.6	258	49	29.5	9.5	6.6	45.9	43.1	68.3
	III	196.7	51.9	3.5	2.2	214	40	23.3	7.2	7.2	45.7	44.9	66.4

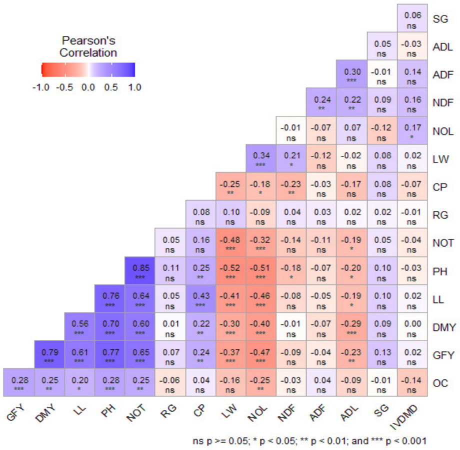

Fig 4.1. Pearson's correlation for GFY and related traits at Ludhiana

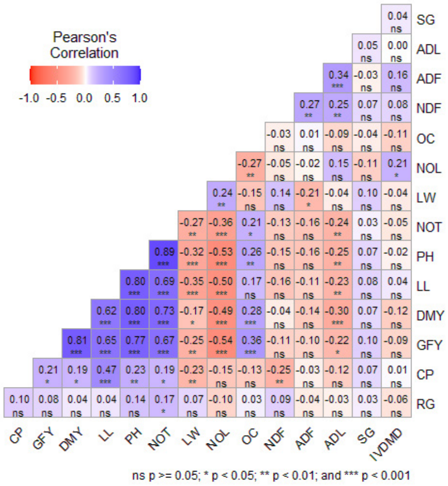

Fig 4.2. Pearson's correlation for GFY and related traits at Gurdaspur

genotypes, which involve interspecific hybridization and recurrent selection.

Cluster analysis: Cluster analysis based on Dice dissimilarity coefficients grouped the 34 genotypes into five major clusters (Fig 5; Table 6), indicating substantial genetic divergence within the germplasm panel. The genetic dissimilarity values ranged from 0.27 to 0.96, suggesting the presence of both closely related and highly divergent genotypes. Such a wide range of genetic distances reflects broad allelic variation and highlights the effectiveness of SSR markers in resolving genetic relationships among polyploid forage grasses. Genotypes within the same cluster exhibited higher genetic similarity, whereas genotypes belonging to different clusters showed pronounced divergence. Similar clustering patterns have been documented in previous studies on Napier grass collections, where genotypes originating from diverse geographical regions and breeding programs were grouped into distinct clusters (Chotiyarnwong *et al*., 2007; Negawo *et al*., 2018; Wanjala *et al*., 2013).

Table 5. Details of polymorphic information content (PIC), alleles amplified and size (bp) of 33 polymorphic SSRs

S. No.	Primer	PIC value	Allele amplified	Size (bp)
1	CTM-10	0.20	4	120
2	CTM-12	0.27	3	280
3	CTM-27	0.22	3	140
4	CTM-59	0.71	3	250
5	CTM-8	0.60	2	100
6	PGIRD13	0.68	4	160
7	PGIRD21	0.37	2	140
8	PGIRD25	0.41	3	125
9	PGIRD5	0.24	3	300
10	PGIRD56	0.40	2	150
11	PGIRD57	0.32	2	120
12	PSMP2235	0.20	2	270
13	PSMP2248	0.79	2	250
14	PSMP2255	0.21	4	200
15	PSMP2266	0.30	5	280
16	PSMP2267	0.33	2	300
17	XTXP27	0.06	4	160
18	Phil227562	0.23	3	180
19	PSMP2227	0.65	3	120
20	PSMP2229	0.60	3	100
21	PSMP2231	0.24	2	250
22	PSMP2232	0.22	2	200
23	PSMP2233	0.19	2	300
24	PSMP2236	0.59	2	270
25	PSMP2237	0.68	3	240
26	PSMP2240	0.60	3	270
27	PSMP2251	0.34	3	250
28	PSMP2253	0.71	2	280
29	PSMP2261	0.23	2	240
30	PSMP2263	0.39	4	200
31	PSMP2271	0.67	2	280
32	PSMP2273	0.76	3	200
33	PSMP2274	0.71	2	260

The genetic differentiation observed among clusters may be attributed to differences in genotype origin, breeding history, microsatellite composition, environmental adaptation and selection pressure. The results clearly demonstrate that SSR-based molecular profiling provides

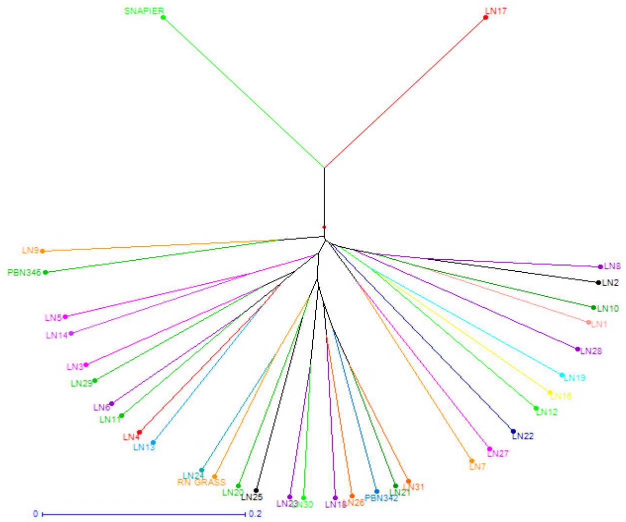

Fig 5. Radial dendrogram obtained by SSR marker analysis using DARwin 6.0

Table 6. Clustering pattern obtained by SSR marker analysis

Cluster	Number of genotypes	Genotypes
I	2	Super napier grass, LN-17
II	2	LN-9, PBN 346
III	8	LN-5, LN-14, LN-3, LN-29, LN-6, LN-11, LN-4, LN-13
IV	11	LN-24, Red napier grass, LN-20, LN-25, LN-23, LN-30, LN-18, LN-26, LN-21, LN-31, PBN 342
V	11	LN-7, LN-27, LN-22, LN-12, LN-16, LN-19, LN-28, LN-11, LN-10, LN-2, LN-8

a reliable framework for genetic diversity assessment, germplasm classification and parental selection in napier grass improvement programs.

Conclusion

The present study aimed to conduct morphological as well as molecular characterization of 34 Napier grass germplasm lines. The comprehensive assessment of the genotypes across two locations will help in future breeding initiatives aimed at enhancing the quality and yield of the fodder crop. Genotypes such as LN-1, LN-6, LN-13, LN-27, LN-28, LN-30, LN-31 and Red Napier grass demonstrated consistent high performance in terms of fodder yield and nutritional quality across locations. Through GGE biplot analysis, significant interactions for various traits were observed. LN-27, LN-13 and LN-31 were stable in Ludhiana, while LN-30 and LN-23 performed well in Gurdaspur. Positive correlations between GFY and traits like PH, NOT/plant and DMY were observed. These traits are crucial for enhancing fodder yield. SSR marker study identified high polymorphism among the genotypes. LN-1, LN-5, LN-6, LN-9, LN-13, LN-16, LN-17, LN-18, Super napier grass, LN-25, LN-27, LN-28, LN-29, LN-30, LN-31 and Red napier grass were identified as demonstrating better performance, showing significant genetic diversity. Overall, the study underscores the potential of diverse napier grass genotypes for breeding programs aimed at improving fodder yield and nutritional quality.

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