

Comparative Grain Morphological Characterization of Indigenous Aromatic, Non-Aromatic and Wild Rice (*Oryza Rufipogon* and *Oryza Nivara*) Germplasm Collected from the Two Valley Districts of Manipur, North-East India and its Implications for Conservation and Pre Breeding

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“Comparative Grain Morphological Characterization of Indigenous Aromatic, Non-Aromatic and Wild Rice (*Oryza Rufipogon* and *Oryza Nivara*) Germplasm Collected from the Two Valley Districts of Manipur, North-East India and its Implications for Conservation and Pre Breeding”

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Abstract

Rice (*Oryza sativa* L.) is one of the most prominent staple crops for human consumption and different grain characters such as grain length and breadth; caryopsis length and breadth, grain shape and colour are crucial for adjudicating rice quality and market value. Although numerous studies have characterised cultivated or wild rice, comprehensive comparative analysis of indigenous aromatic, non-aromatic and wild rice germplasm -*Oryza rufipogon* (Wainuchara) and *Oryza nivara* (Murshi) from Manipur, Northeast India remain limited. Therefore, the present study represents the comparative grain morphological analysis and multivariate statistical assessment of some promising selected rice accessions. Twenty-three accessions were characterized for ten grain traits, followed by descriptive statistics, Analysis of Variance (ANOVA) with Tukey's HSD, Pearson correlation, principal component analysis (PCA) and hierarchical clustering. Considerable variation was observed in 100-grain weight (1.552–3.011 g), grain length (6.06–9.83 mm), grain width (2.10–3.60 mm), grain thickness (1.50–2.36 mm) and L/B ratio (1.91–4.00). Very Long–Medium grains predominated (52.17%). Grain thickness differed significantly among groups ($F = 4.090$, $P = 0.032$), while awn length was the strongest discriminator ($F = 42.892$, $P < 0.001$), with wild rice differing significantly from aromatic and non-aromatic rice. PCA showed that PC1 (eigenvalue = 4.242) and PC2 (2.045) explained 53.024% and 25.568% of variation, respectively, accounting for 78.592% cumulatively. PCA separated the accessions into two groups: Cluster I comprised all ten wild accessions plus Taothabi (NRM10), whereas Cluster II contained the remaining cultivated accessions. Hierarchical clustering similarly produced two major clusters at a rescaled distance of approximately 25, with 11 accessions in the wild-associated cluster and 12 predominantly cultivated accessions. The concordance between PCA and clustering confirms strong cultivated–wild differentiation and highlights Wainuchara and Murshi as valuable sources of distinctive grain and awn characteristics. These germplasm resources have potential for germplasm conservation, pre-breeding, broadening the cultivated rice gene pool and developing varieties with improved grain quality and adaptation.

Keywords: Rice; Wild rice; aromatic rice; non-aromatic rice; Morphological analysis; multivariate statistical assessment; Germplasm conservation;

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important staple food crops and plays a central role in global food and nutritional security. China remains the world's largest rice-producing country, while India ranks second and is also a major rice-exporting nation. In India, rice production has increased substantially in recent years, reaching approximately 149.1 million tonnes in 2024–25, with rice cultivated over about 51.42 million hectares and an average productivity of approximately 2.90 tonnes ha⁻¹ (ICAR, 2025). The increasing demand for high-quality rice, coupled with the challenges posed by climate change, declining genetic diversity, and emerging biotic and abiotic stresses, has intensified the need for the conservation and utilization of indigenous rice germplasm in crop improvement programmes (FAO, 2024; IRRI, 2024). Consequently, characterization and evaluation of indigenous rice germplasm are essential for identifying valuable traits that can contribute to future rice improvement programmes (ICAR, 2025). Manipur, a northeastern state of India, is a rich reservoir of rice genetic diversity, harbouring native aromatic, non-aromatic, wild and pigmented (black, red, and purple) rice types owing to its diverse agro-climatic conditions, traditional farming systems, and long history of farmer selection. Black rice (Chakhao) is traditionally thought to have therapeutic properties, it holds a special place in the Indian rice market. Many of these traditional varieties are highly valued for their unique culinary qualities and cultural importance, while several remain underutilized and poorly characterized despite their potential significance in rice breeding and genetic conservation (Hore, 2005; Medhabati *et al.*, 2013). Numerous wild relatives of cultivated rice collected from diverse ecological zones across India constitute a valuable reservoir of genetic diversity, having genes that confer resistance to major biotic stresses such as blast, brown planthopper, and grassy stunt virus, and have historically served as important donors of disease- and pest-resistance traits for the genetic improvement of *Oryza sativa* (Brar & Khush, 1997; Patra *et al.*, 2008; Singh *et al.*, 2013, 2018). Rice grain size and shape are major determinants of grain quality, influencing consumer preference, milling efficiency, market value, and cooking characteristics, and are controlled by multiple genes and quantitative trait loci (QTLs), including GS3, GW2, GW5 (qSW5), GL7, and qSW5 (Fan *et al.*, 2006; Song *et al.*, 2007; Shomura *et al.*, 2008; C. Wang *et al.*, 2011). Consequently, characterization of grain morphological diversity provides valuable information for selecting parental lines in breeding programmes aimed at improving grain quality and yield while meeting consumer preferences (Li *et al.*, 2021; Zeng *et al.*, 2022). Although several studies have investigated rice grain quality and morphological diversity, comparative study using statistical analysis of indigenous aromatic, non-aromatic, and wild rice germplasm from the Manipur valley remains limited. Therefore, the present study characterizes and compares grain morphological traits, including grain length, grain width, length-to-breadth ratio, grain shape, caryopsis length and breadth, rice bran colour and 100-grain weight, using standardized descriptors and statistical analysis like coefficient of variation (CV), ANOVA, Principal Component Analysis (PCA) and hierarchical cluster analysis using IBM SPSS Statistics version 21.0 to identify phenotypic variation and promising donor accessions. The findings provide baseline information for identifying promising donor accessions for future pre-breeding, conservation, characterization, and utilization of Manipur rice genetic resources in breeding high-quality and climate-resilient rice cultivars.

MATERIALS AND METHODS

Rice Sample Preparation

A total of 23 indigenous rice accessions comprising three aromatic landraces, ten non-aromatic landraces, and ten wild rice accessions were included in the present investigation (Supplementary Table 1). Thirteen cultivated rice accessions were collected during the Kharif seasons of 2024 and 2025 from the Rice Research Station, Wangbal, Thoubal District, Manipur, India (24°36'51.4" N, 94°01'18.9" E), whereas the ten wild rice accessions of Murshi (*Oryza nivara* Sharma & Shastri) and Wainuchara (*Oryza rufipogon* Griff.), were collected from the natural populations growing along the lakeshore of Ikop Paat (24°35'02.8"N 93°56'40.1"E), Waithou Paat(24°40'56.0"N 93°58'38.4"E), Thoubal District and Loktak Paat(24°31'54.1"N 93°48'56.5"E), Bishnupur District, Manipur. Ex-situ cultivation of two wild rice accessions namely WCM006 and MUR004 were also carried out at Lilong(24°42'03.4"N 93°55'03.6"E), Thoubal District, Manipur. Passport information, including accession name, collection site, habitat, and local identity, was recorded during sample collection. All plant materials were authenticated at the Department of Botany, Dhanamanjuri University, Imphal, India.

Freshly harvested mature panicles were collected after physiological maturity and the grains were manually cleaned to remove inert materials, immature seeds, and damaged grains. The cleaned samples were air-dried to a moisture content of approximately 12–14% under ambient laboratory conditions before storage. All samples were stored in airtight polyethylene containers at 25 ± 2 °C until morphological evaluation. Before grain characterization, the paddy grains were manually dehulled to obtain brown rice (decorticated grains), ensuring that the grains remained intact without mechanical damage.

Grain Morphological Characterization

Grain morphological characterization was carried out following the Bioversity International–IRRI–WARDA Rice Descriptor (2007) and the standard procedures described by Roy and Sharma (2014). For each accession, 100 well-filled grains were randomly selected for analysis. Grain morphological traits were evaluated using ten independent replications, and the mean values were used for statistical analysis. Grain quality traits of biological replica of each accession were recorded. The weight of 100 fully developed grains was measured in grams (gm) using a digital analytical balance (accuracy ± 0.001 gm). The length of dehulled grains was measured using a digital Vernier calliper (Mitutoyo, Japan; precision 0.01 mm). Grain length was measured from the base to the apex of each grain, excluding the awn. Grain width and thickness was measured at the widest portion of each grain using the same digital Vernier calliper. The grain length-to-breadth ratio was also calculated. Grain size and shape were classified following the IRRI Standard Evaluation System (SES) for Rice (2013) and Bioversity International–IRRI–WARDA (2007) based on grain length and length-to-breadth ratio. Grain length was subsequently categorized into short (<5.50 mm), medium (5.51–6.60 mm), long (6.61–7.50 mm) and very long (>7.50 mm) classes following the conventional IRRI grain-length classification. Grain shape was calculated as the length-to-width ratio and classified as slender (>3.0), medium (2.1–3.0) and bold (<2.1). Caryopsis length and breadth were also measured from the decorticated grain and rice bran colour were also recorded.

Statistical Analysis

All recorded data were subjected to statistical analysis using IBM SPSS Statistics version 21.0. Descriptive statistics, including mean, standard deviation (SD), ranges and coefficient of variation (CV), were computed for each grain trait for each replica of each accession. Differences among three groups of rice accessions i.e. aromatic, non-aromatic and wild rice, were evaluated using one-way analysis of variance (ANOVA). Pearson's correlation coefficients were calculated to determine the relationships among grain morphological traits. In addition, Principal Component Analysis (PCA) was performed using standardized data to identify the major traits contributing to phenotypic variation among the accessions using SPSS version 21.0. Hierarchical Cluster Analysis (HCA) based on Euclidean distance and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was carried out to evaluate the morphological relationships among the rice accessions. Each accession was assigned a unique reference code for proper documentation and data management. The identification codes of aromatic, non-aromatic, and wild rice accessions are presented in Supplementary Table 1.

RESULTS AND DISCUSSION

Variation in grain morphological characteristics among rice accessions

Twenty-three rice accessions comprising aromatic, non-aromatic, *Oryza rufipogon* (Wainuchara), and *Oryza nivara* Sharma & Shastry (Murshi) were evaluated for ten important grain quality parameters, namely 100-grain weight, grain length, grain width, grain thickness, length-to-breadth (L/B) ratio, caryopsis length, caryopsis breadth, bran colour, awn length and grain shape (Table 1). Descriptive statistics are presented in Table 2. The Test weight (100-grain weight) exhibits considerable variation, ranging from lowest grain weight in Chakhao Khongnembi (1.552 gm) to highest grain weight in Lang Phou (3.011 gm), with an overall mean of 2.05 gm, standard deviation (SD) of 0.268, and coefficient of variation (CV) of 13.07%, indicating moderate variability among the accessions. The grain length varies from shortest in Heimang Phou (6.06 mm) to longest in Lang Phou (9.83 mm), with an average value of 8.58 mm, SD of 0.796 and CV of 9.28%. The grain width ranges from the narrowest grains records in a Wainuchara accession i.e. WCM002(2.10 mm) to broadest grain in the accession of Murshi i.e. MUR002 (3.60 mm), with a mean of 2.91 mm, SD of 0.431 mm, and CV of 14.80%. Grain thickness ranges from the highest in Lang Phou (2.36 mm) to the lowest value in WCM003 (1.50 mm) , with a mean of 1.99 and a CV of 13.10%. The L/B ratio showed the highest variation among the major grain-dimension traits, ranging from the highest in the accessions of Wainuchara i.e. WCM002 (4.00), followed by other accessions like WCM003 (3.33), WCM005 (3.64), WCM006 (3.58) and WCM004 (3.52) to the lowest L/B ratio (1.91) in Heimang Phou (NRM01), with a mean of 3.00, SD of 0.526 mm and a CV of 17.54. Based on grain morphology, grain shape is calculated based on grain length (short <5.50 mm, medium= 5.51–6.60 mm, long= 6.61–7.50 mm and very long >7.50 mm) and length-to-width ratio (slender >3.0, medium= 2.1–3.0 and bold <2.1). The Very Long–Medium category is the predominant grain type, comprising 12 of the 23 accessions (52.17%). The accessions belonging to this group are Chakhao amubi (L=8.59 mm; L/B= 2.77), Chakhao khongnembi ARM03 (8.62 mm; 2.67), Kakcheng Phou (7.90 mm; 2.58), Lang Phou (9.83 mm; 2.84), Phoudum (8.23 mm; 2.71), Phougak (8.03 mm; 2.59), Sangsangba (8.40 mm; 2.52), Taothabi (8.38 mm; 2.72), and all the accessions of Murshi-MUR001 (9.10 mm; 2.93), MUR002 (9.60 mm; 2.67), MUR003 (8.90 mm; 2.61) and

MUR004 (8.00 mm; 2.35). The Very Long–Slender group comprised 10 accessions (43.48%), making it the second major grain class. This group included Langphou Chakhao (L= 8.90 mm; L/B 3.03), Kumbi Phou (9.44 mm; 3.32), Phouren (9.28 mm; 3.59), Phouren Amubi (9.60 mm; 3.35) and all the accessions of Wainuchara- WCM001 (8.30 mm; 3.77), WCM002 (8.40 mm; 4.00), WCM003 (8.00 mm; 3.33), WCM004 (8.10 mm; 3.52), WCM005 (9.10 mm; 3.64) and WCM006 (8.60 mm; 3.58). In contrast, no accession (0.00%) belonged to the Long–Medium category. 95.65% of the accessions (22 out of 23) belonged to either the Very Long–Medium or Very Long–Slender categories. Caryopsis length varies from the longest caryopsis (7.30 mm) in Phouren (NRM07) and the shortest (4.40 mm) in Heimang Phou (NRM01), with an average of 6.39, SD of 0.687 and a CV of 10.77%. Caryopsis width ranged from the maximum caryopsis width (2.91 mm) in Heimang Phou, whereas two accessions of Wainuchara i.e. WCM001 and WCM005 recorded the minimum width (1.80 mm), with a mean of 2.41, SD of 0.379 and a CV of 15.75%. Awn length showed the largest magnitude of variation among all quantitative characters. Values ranged from 0 to 52.0 mm, with a mean of 18.35, SD of 20.24, variance of 409.695 and an exceptionally high CV of 110.33%. The absence of awns in several accessions and the presence of comparatively long awns in others indicate strong phenotypic differentiation for this character. An accession of Wainuchara (WCM006) recorded an awn length of 52.0 mm, followed by other accessions like WCM005 (50.0 mm), WCM001 (40.5 mm), WCM003 (40.4 mm), MUR004 (39.0 mm), WCM002 (38.0 mm), MUR002 (37.4 mm) and NRM10 (35.64 mm). In contrast, the aromatic and most non-aromatic accession, except Taothabi, lacked measurable awns. Qualitative variation was also observed for rice bran colour. The accessions includes white, reddish-brown, dark red, brown, light brown and red bran colour categories. Chakhao amubi(ARM01) and Sangsangba(NRM09) possess dark-red bran; Chakhao khongnembi(ARM03), Phougak(NRM06) and Taothabi(NRM10) shows reddish-brown colour; aromatic accessions like ARM02, non-aromatic accessions like NRM01,NRM02, NRM03,NRM04, NRM05, NRM07 and NRM08 shows white rice bran; while the Murshi accessions consistently display red bran. The Wainuchara accessions are predominantly brown or light brown.



Fig.1: Selected Aromatic rice found in the valley of Manipur- a)Chakhao Amubi, b)Langphou Chakhao and c) Chakhao khongnembi

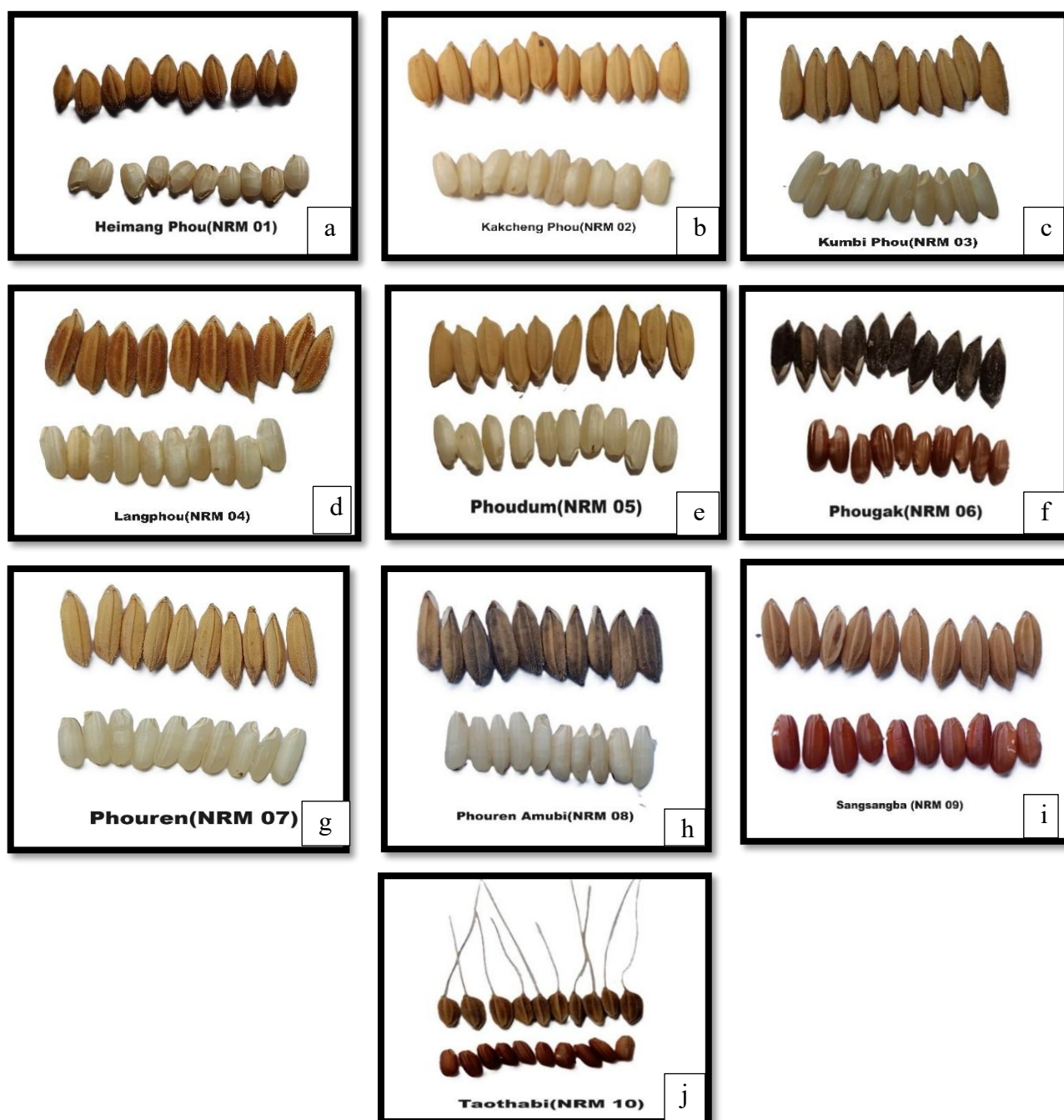


Fig. 2 Selected non-Aromatic rice found in the valley of Manipur- a) Heimang Phou, b) Kakcheng Phou, c) Kumbi Phou, d) Lang Phou, e) Phoudum, f) Phougak, g) Phouren, h) Phouren Amubi, i) Sansangba, j) Taothabi

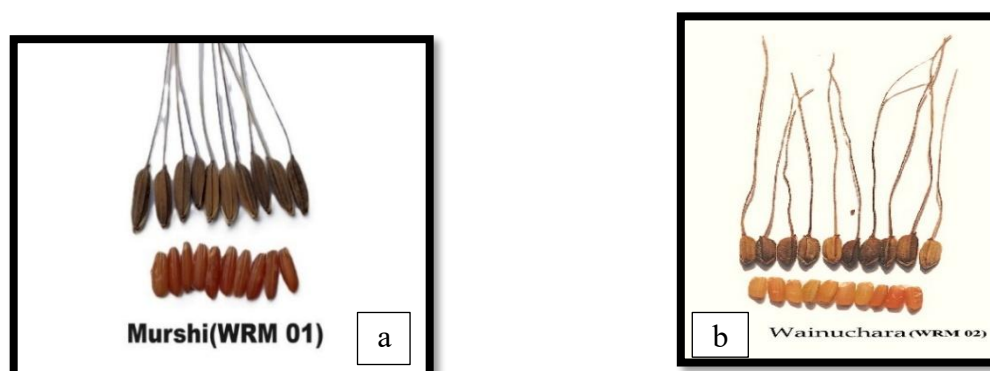


Fig. 3 Two wild rice found in the valley of Manipur- a) Murshi and b) Wainuchara

Table 1 Variation in quantitative and qualitative grain morphological characteristics among the 23 rice accessions

Sl. No	Code	Test weight (100 grain weight)	Grain length (mm)	Grain Breadth (mm)	Grain Thickness (mm)	L/B	Caryopsis length	Caryopsis width	Awn Length (mm)	Rice brar colour	Grain Shape
1	ARM01	2.189	8.59	3.10	2.06	2.77	6.30	2.60	-	Dark Red	Very Long-medium
2	ARM02	1.926	8.90	2.93	2.06	3.03	6.45	2.51	-	White	Very Long-slender
3	ARM03	1.552	8.62	3.22	2.14	2.67	6.00	2.50	-	Reddish Brown	Very Long-medium
4	NRM01	1.699	6.06	3.16	2.33	1.91	4.40	2.91	-	White	Long-medium
5	NRM02	2.112	7.9	3.06	2.26	2.58	6.12	2.90	-	White	Very Long-medium
6	NRM03	2.153	9.44	2.84	2.00	3.32	6.92	2.30	-	White	Very Long-slender
7	NRM04	3.011	9.83	3.46	2.36	2.84	7.20	2.80	-	White	Very Long-medium
8	NRM05	2.05	8.23	3.03	2.2	2.71	5.53	2.50	-	White	Very Long-medium
9	NRM06	1.834	8.03	3.1	1.96	2.59	5.81	2.50	-	Reddish Brown	Very Long-medium
10	NRM07	1.895	9.28	2.58	1.88	3.59	7.30	2.20	-	White	Very Long-slender
11	NRM08	2.123	9.60	2.86	2.23	3.35	7.15	2.22	-	White	Very Long-slender
12	NRM09	2.325	8.40	3.33	2.06	2.52	6.10	2.81		Dark red	Very Long-medium
13	NRM10	2.119	8.38	3.08	1.98	2.72	5.75	2.52	35.64	Reddish Brown	Very Long-medium
14	WCM001	2.11	8.30	2.20	1.60	3.77	6.60	1.80	40.50	Brown	Very Long-slender
15	WCM002	2.10	8.40	2.10	1.60	4.00	7.00	1.90	38.00	Light brown	Very Long-slender
16	WCM003	2.10	8.00	2.40	1.50	3.33	6.20	1.90	40.40	Light brown	Very Long-slender
17	WCM004	2.05	8.10	2.30	1.70	3.52	6.70	2.10	28.50	Light brown	Very Long-slender
18	WCM005	2.01	9.10	2.50	1.60	3.64	6.60	1.80	50.00	Brown	Very Long-slender
19	WCM006	1.99	8.60	2.40	1.70	3.58	6.80	1.80	52.00	Brown	Very Long-slender
20	MUR001	1.90	9.10	3.10	2.10	2.93	7.00	2.50	31.00	Red	Very Long-medium
21	MUR002	1.85	9.60	3.60	2.10	2.67	7.20	2.80	37.40	Red	Very Long-medium
22	MUR003	2.00	8.90	3.40	2.20	2.61	6.20	2.80	29.50	Red	Very Long-medium
23	MUR004	2.05	8.00	3.40	2.30	2.35	5.70	2.80	39.00	Red	Very Long-medium

Grain shape is calculated based on Grain length(short <5.50 mm, medium= 5.51–6.60 mm, long= 6.61–7.50 mm and very long >7.50 mm) and length-to-width ratio (slender >3.0, medium= 2.1–3.0 and bold <2.1).

Table 2. Descriptive statistics and phenotypic variability of grain morphological traits among 23 rice accessions

Traits	N Statistic	Minimum	Maximum	Mean		Std. Deviation	Variance	CV (%)
				Statistic	Std. Error			
Test weight	23	1.552	3.011	2.04991	.055863	.267910	.072	13.07
Grain length	23	6.06	9.83	8.58	.166	.796	.634	9.28
Grain Breath	23	2.10	3.60	2.9196	.09005	.43186	.187	14.80
Grain Thickness	23	1.50	2.36	1.9965	.05455	.26160	.068	13.10
L/B	23	1.91	4.00	3.0000	.10974	.52628	.277	17.54
Caryopsis length	23	4.40	7.30	6.3926	.14343	.68788	.473	10.77
Caryopsis width	23	1.80	2.91	2.4117	.07920	.37983	.144	15.75
Awn Length	23	0	52	18.35	4.221	20.241	409.695	110.33

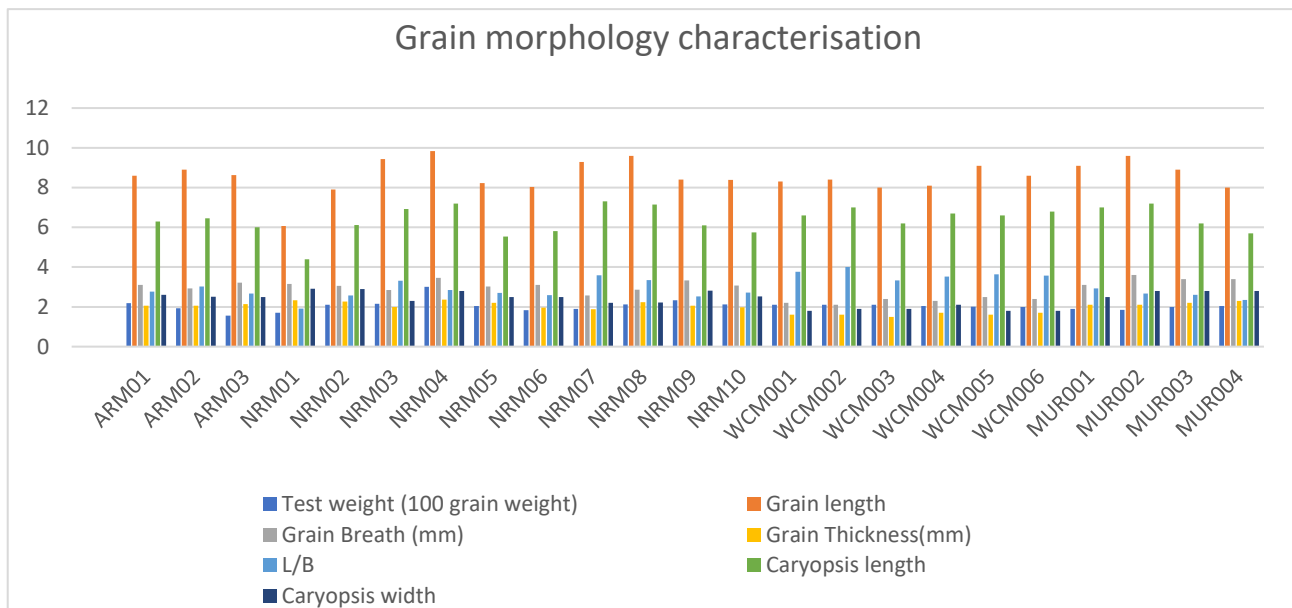


Fig 4. Bar chart showing variation of grain morphological characters among the selected 23 rice accessions

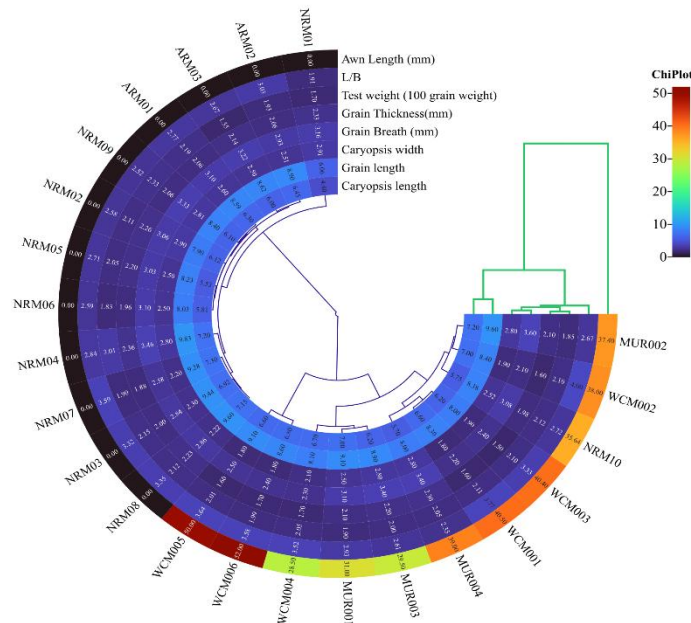


Fig 5. Circular heatmap and UPGMA dendrogram illustrating the phenotypic relationships among 23 rice accessions based on eight grain morphological traits.

Correlations

The accession-wise Pearson's Correlations Coefficients similarity analysis performed among the 23 wild rice accessions reveals distinct differences between cultivated and wild rice groups (Table 3). Similarity coefficients among the cultivated rice accessions are generally high, ranging from 0.9214 to 0.9995, indicating remarkable phenotypic similarity among cultivated genotypes. The *Oryza rufipogon* accessions (WCM001–WCM006) exhibits very high mutual similarity, with coefficients ranging from 0.9925 to 0.9999, while the *O. nivara* accessions (MUR001–MUR004) shows similarity coefficients ranging from 0.9943 to 0.9995. In contrast, cultivated rice accessions exhibits primarily negative similarity coefficients with the wild-rice accessions, ranging approximately from –0.218 to –0.510, demonstrating clear differentiation between cultivated and wild rice groups. The exceptionally high within-group similarity and dissimilar between-group coefficients indicate considerable group-specific phenotypic differentiation among the evaluated rice accessions.

Table 3. Pearson Correlation coefficients between the selected rice accessions based on grain morphological characters

	ARM01	ARM02	ARM03	NRM01	NRM02	NRM03	NRM04	NRM05	NRM06	NRM07	NRM08	NRM09	NRM10	WCM001	WCM002	WCM003	WCM004	WCM005	WCM006	MUR001	MUR002	MUR003	MUR004
ARM01		0.99837	0.99581	0.96448	0.99704	0.99553	0.997	0.99715	0.9989	0.98819	0.99438	0.99802	-0.34068	-0.35174	-0.33377	-0.36031	-0.27735	-0.37275	-0.38498	-0.27195	-0.30682	-0.27947	-0.36908
ARM02			0.99626	0.95546	0.99384	0.99829	0.99251	0.99602	0.99746	0.99365	0.9976	0.99321	-0.31961	-0.32913	-0.31063	-0.33838	-0.25405	-0.35078	-0.36314	-0.25002	-0.28579	-0.25836	-0.34874
ARM03				0.97029	0.99316	0.99038	0.98791	0.99676	0.99847	0.98239	0.98996	0.99374	-0.31922	-0.33232	-0.31476	-0.34063	-0.2582	-0.35248	-0.36499	-0.25048	-0.28476	-0.25688	-0.34709
NRM01					0.97678	0.93815	0.95761	0.96982	0.97144	0.92143	0.93693	0.97502	-0.46382	-0.48362	-0.4691	-0.4889	-0.41654	-0.49935	-0.51048	-0.40209	-0.4304	-0.40404	-0.48604
NRM02						0.98792	0.99305	0.99334	0.99685	0.98087	0.98722	0.99724	-0.37721	-0.38914	-0.3714	-0.39728	-0.31545	-0.40965	-0.42121	-0.30883	-0.34271	-0.31614	-0.4041
NRM03							0.9913	0.99149	0.99253	0.99724	0.99949	0.98782	-0.30308	-0.31031	-0.29134	-0.32019	-0.2348	-0.33283	-0.34521	-0.23298	-0.26975	-0.24256	-0.33314
NRM04								0.99314	0.9934	0.9813	0.99016	0.99654	-0.34063	-0.35213	-0.33456	-0.36038	-0.27833	-0.37296	-0.38513	-0.27273	-0.30739	-0.27998	-0.36894
NRM05									0.99755	0.98073	0.9903	0.99588	-0.35549	-0.36823	-0.35108	-0.37649	-0.29541	-0.38812	-0.40082	-0.28866	-0.32286	-0.29458	-0.38374
NRM06										0.98458	0.99137	0.99786	-0.34204	-0.35436	-0.33669	-0.36261	-0.28031	-0.37479	-0.38709	-0.27355	-0.30779	-0.28036	-0.36994
NRM07											0.9974	0.97702	-0.29032	-0.29441	-0.27454	-0.30514	-0.2181	-0.31812	-0.33002	-0.21878	-0.25647	-0.23041	-0.32069
NRM08												0.98589	-0.30178	-0.30869	-0.28967	-0.31873	-0.23312	-0.33131	-0.34351	-0.23121	-0.26817	-0.24119	-0.33164
NRM09													-0.35908	-0.37268	-0.3555	-0.38029	-0.29939	-0.39259	-0.40477	-0.29178	-0.32522	-0.29778	-0.38645
NRM10														0.99864	0.99796	0.99909	0.99585	0.99888	0.99822	0.99704	0.99908	0.99779	0.99936
WCM001															0.99972	0.99985	0.99956	0.99923	0.99563	0.99716	0.99499	0.99771	
WCM002																0.99923	0.99822	0.99861	0.9981	0.9966	0.99721	0.99541	0.99634
WCM003																	0.99582	0.99986	0.99962	0.99521	0.99736	0.99504	0.99862
WCM004																		0.99438	0.99314	0.99886	0.99736	0.9972	0.99253
WCM005																			0.99986	0.9939	0.99658	0.99402	0.99883
WCM006																				0.99252	0.99564	0.99255	0.99865
MUR001																					0.99913	0.99948	0.99428
MUR002																						0.99943	0.99769
MUR003																							0.99549
MUR004																							

Pearson correlation analysis among rice accessions based on morphological grain characters was performed to determine the degree of similarity among the 23 rice accessions (Table 4). Pearson correlation analysis unveils significant associations among several grain morphological traits. Grain length shows a strong positive correlation with grain breadth ($r = 0.860$, $P < 0.01$) and caryopsis length ($r = 0.916$, $P < 0.01$), whereas grain length is strongly negatively correlated with grain thickness ($r = -0.863$, $P < 0.01$). Grain breadth demonstrates a significant negative correlation with grain thickness ($r = -0.796$, $P < 0.01$) and a strong positive correlation with caryopsis length ($r = 0.891$, $P < 0.01$). A significant negative association is also observed between grain breadth and caryopsis width ($r = -0.606$, $P < 0.01$). Grain thickness shows a very strong negative correlation with L/B ratio ($r = -0.907$, $P < 0.01$). Similarly, L/B ratio exhibits a significant positive correlation with caryopsis length ($r = 0.695$, $P < 0.01$) and a very strong negative correlation with caryopsis width ($r = -0.907$, $P < 0.01$). These results demonstrate strong interrelationships among

grain dimensions, particularly among grain length, breadth, thickness, L/B ratio and caryopsis dimensions. In contrast, test weight shows no significant correlation with the measured grain morphological traits, with correlation coefficients ranging from -0.083 to 0.369 . Therefore, the similarity analysis clearly differentiates cultivated and wild rice groups, whereas the Pearson correlation analysis identifies grain dimensions and caryopsis traits as the major interrelated components of grain morphology. The strong and significant correlations observe among these traits suggest that selection for particular grain dimensions may have correlated effects on other components of grain shape and size.

Table 4. Pearson correlation coefficients among quantitative grain morphological traits of the 23 rice accessions

	Pearson Similarity Correlation Matrix							
	Test weight	Grain length	Grain Breath	Grain Thickness	L/B	Caryopsis length	Caryopsis width	Awn Length
Test weight		.369	.076	.102	.124	.336	.074	-.083
Grain length			.101	.002	.405	.853	-.144	.017
Grain Breath				.860	-.863	-.281	.916	-.402
Grain Thickness					-.796	-.307	.891	-.606
L/B						.695	-.907	.419
Caryopsis length							-.410	.188
Caryopsis width								-.523
Awn Length								

One-Way ANOVA

One-way analysis of variance shows significant differences among aromatic, non-aromatic, and wild rice groups for grain length and grain width, whereas 100-grain weight and the length-to-breadth (L/B) ratio shows no significant differences (Table 5). One-way analysis of variance reveals significant differences among aromatic, non-aromatic and wild rice accessions for grain thickness and awn length, whereas test weight, grain length, grain breadth, L/B ratio, caryopsis length and caryopsis width did not differ significantly among the three rice groups. Grain thickness showed a significant group effect ($F = 4.090$, $P = 0.032$), while awn length exhibited a highly significant difference ($F_2 = 42.892$, $P < 0.001$). In contrast, the effects of group were non-significant for test weight ($F = 1.102$, $P = 0.352$), grain length ($F = 0.070$, $P = 0.933$), grain breadth ($F = 1.623$, $P = 0.222$), L/B ratio ($F = 2.009$, $P = 0.160$), caryopsis length ($F = 0.790$, $P = 0.468$) and caryopsis width ($F = 2.587$, $P = 0.100$).

Tukey's HSD post-hoc test further demonstrates that the significant variation in grain thickness is attributable specifically to the difference between non-aromatic and wild rice accessions (Table 6). The mean difference between these groups is 0.286 , with a standard error of 0.103 , and is statistically significant ($P = 0.031$; 95% Ci: 0.0245 – 0.5475). No significant difference is detected between aromatic and non-aromatic accessions ($P = 0.964$) or between aromatic and wild accessions ($P = 0.260$). For awn length, Tukey's HSD reveals highly significant differences between aromatic and wild rice (mean difference = -38.630 , $P < 0.001$; 95% Ci: -54.00 to -23.26) and between non-aromatic and wild rice

(mean difference = -35.066 , $P < 0.001$; 95% Ci: -45.51 to -24.62). However, the difference between aromatic and non-aromatic rice is non-significant (mean difference = -3.564 , $P = 0.829$; 95% Ci: -18.94 to 11.81). Thus, the highly significant variation in awn length is primarily associated with the distinct morphology of the wild rice group relative to both cultivated rice groups. Hence, the ANOVA and Tukey HSD results demonstrate that grain thickness and particularly awn length were effective discriminatory traits among the three rice groups, with awn length providing the strongest evidence of phenotypic differentiation ($P < 0.001$). The remaining grain morphological traits shows no statistically significant group-level differences ($P > 0.05$).

Table 5. One-way ANOVA among aromatic, non-aromatic and wild rice accessions based on the grain morphological characters

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.(p)
Test weight	Between Groups	.157	2	.078	1.102	.352(ns)
	Within Groups	1.422	20	.071		
	Total	1.579	22			
Grain length	Between Groups	.097	2	.049	.070	.933(ns)
	Within Groups	13.844	20	.692		
	Total	13.941	22			
Grain Breadth	Between Groups	.573	2	.287	1.623	.222(ns)
	Within Groups	3.530	20	.177		
	Total	4.103	22			
Grain Thickness	Between Groups	.437	2	.219	4.090	.032(s)
	Within Groups	1.069	20	.053		
	Total	1.506	22			
L/B	Between Groups	1.019	2	.510	2.009	.160(ns)
	Within Groups	5.074	20	.254		
	Total	6.093	22			
Caryopsis length	Between Groups	.762	2	.381	.790	.468(ns)
	Within Groups	9.648	20	.482		
	Total	10.410	22			
Caryopsis width	Between Groups	.652	2	.326	2.587	.100(ns)
	Within Groups	2.522	20	.126		
	Total	3.174	22			
Awn Length	Between Groups	7309.209	2	3654.604	42.892	<.001(hs)
	Within Groups	1704.090	20	85.204		
	Total	9013.299	22			

(ns= not significant ($p > 0.05$), s= significant, hs= highly significant)

Table 6 Tukey's HSD pairwise comparisons of grain morphological traits among aromatic, non-aromatic and wild rice groups

Tukey HSD

Dependent Variable	(I) Code	(J) Code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval(Ci)	
						Lower Bound	Upper Bound
Test weight	1	2	-.243100	.175549	.367	-.68724	.20104
		3	-.127000	.175549	.753	-.57114	.31714
		1	.243100	.175549	.367	-.20104	.68724
	2	3	.116100	.119262	.602	-.18563	.41783
		1	.127000	.175549	.753	-.31714	.57114
		2	-.116100	.119262	.602	-.41783	.18563
Grain length	1	2	.187	.548	.938	-1.20	1.57
		3	.090	.548	.985	-1.30	1.48
		1	-.187	.548	.938	-1.57	1.20
	2	3	-.097	.372	.963	-1.04	.84
		1	-.090	.548	.985	-1.48	1.30
		2	.097	.372	.963	-.84	1.04
Grain Breadth	1	2	.03333	.27656	.992	-.6664	.7330

Grain Thickness	2	3	.34333	.27656	.444	-.3564	1.0430
		1	-.03333	.27656	.992	-.7330	.6664
		3	.31000	.18788	.249	-.1653	.7853
	3	1	-.34333	.27656	.444	-1.0430	.3564
		2	-.31000	.18788	.249	-.7853	.1653
	1	2	-.03933	.15215	.964	-.4243	.3456
		3	.24667	.15215	.260	-.1383	.6316
		1	.03933	.15215	.964	-.3456	.4243
	2	3	.28600*	.10337	.031	.0245	.5475
L/B	3	1	-.24667	.15215	.260	-.6316	.1383
		2	-.28600*	.10337	.031	-.5475	-.0245
		1	.01033	.33157	.999	-.8285	.8492
	1	3	-.41667	.33157	.435	-1.2555	.4222
		1	-.01033	.33157	.999	-.8492	.8285
	2	3	-.42700	.22526	.166	-.9969	.1429
		1	.41667	.33157	.435	-.4222	1.2555
	3	2	.42700	.22526	.166	-.1429	.9969
		2	.02200	.45721	.999	-1.1347	1.1787
Caryopsis length	1	3	-.35000	.45721	.728	-1.5067	.8067
		1	-.02200	.45721	.999	-1.1787	1.1347
		3	-.37200	.31061	.468	-1.1578	.4138
	3	1	.35000	.45721	.728	-.8067	1.5067
		2	.37200	.31061	.468	-.4138	1.1578
	1	2	-.02933	.23374	.991	-.6207	.6207
		3	.31667	.23374	.383	-.2747	.9080
		1	.02933	.23374	.991	-.5620	.6207
	2	3	.34600	.15879	.099	-.0557	.7477
Caryopsis width	3	1	-.31667	.23374	.383	-.9080	.2747
		2	-.34600	.15879	.099	-.7477	.0557
		2	-3.564	6.076	.829	-18.94	11.81
	1	3	-38.630*	6.076	.000	-54.00	-23.26
		1	3.564	6.076	.829	-11.81	18.94
	2	3	-35.066*	4.128	.000	-45.51	-24.62
		1	38.630*	6.076	.000	23.26	54.00
	3	2	35.066*	4.128	.000	24.62	45.51

*. The mean difference is significant at the 0.05 level.

(Code: 1= aromatic rice accessions, 2= non-aromatic rice accessions, 3= wild rice accessions)

Principal Component Axis (PCA)

Principal Component Analysis (PCA) was performed to assess the pattern of variation and morphological relationships among the 23 rice accessions based on eight grain physical characteristics, namely 100-grain weight, grain length, grain width, length-to-breadth (L/B) ratio, caryopsis length, caryopsis breadth and awn length (Figure 5). The PCA component plot separates the rice accessions into two distinct groups. Cluster I comprised all ten wild-rice accessions (WCM001–WCM006 and MUR001–MUR004) together with NRM10, characterize by negative Component 1 scores (approximately -0.40 to 0.00) and high positive Component 2 scores (approximately 0.90–1.00). Cluster II comprises the remaining cultivated accessions (ARM01–ARM03 and NRM01–NRM09), characterized by positive Component 1 scores (approximately 0.90–1.00) and negative to near-zero Component 2 scores (approximately -0.35 to 0.00). Principal component analysis of the eight grain morphological traits reveals that the first two principal components accounted for 78.592% of the total variation (Figure 6). PC1 has an eigenvalue of 4.242 and explained 53.024% of the total variance, whereas PC2 has an eigenvalue of 2.045 and accounted for 25.568%. The remaining components contributed comparatively smaller proportions of variation: PC3 = 9.265%, PC4 = 8.790%, PC5 = 1.635%, PC6 = 1.395%, PC7 = 0.281%, and PC8 = 0.041%. The cumulative variance reached 96.647% by PC4 and 100.00% by PC8. The

component plot indicated that grain thickness, grain breadth and caryopsis width were strongly associated with the positive direction of PC1, whereas L/B ratio and awn length were positioned in the negative direction of PC1. Along PC2, grain length and caryopsis length showed positive associations, together with test weight/100-grain weight. Thus, PC1 primarily represented variation associated with grain breadth, thickness and caryopsis width versus L/B ratio and awn length, while PC2 was largely associated with grain length, caryopsis length and grain weight. The scree plot showed a pronounced decline in eigenvalues from PC1 (4.242) to PC2 (2.045), followed by a substantial reduction at PC3 (0.741) and PC4 (0.703). Thereafter, eigenvalues declined sharply to 0.131, 0.112, 0.023 and 0.003 for PC5–PC8, respectively. The scree pattern therefore indicates that PC1 and PC2 were the major components contributing to the observed grain morphological variation.

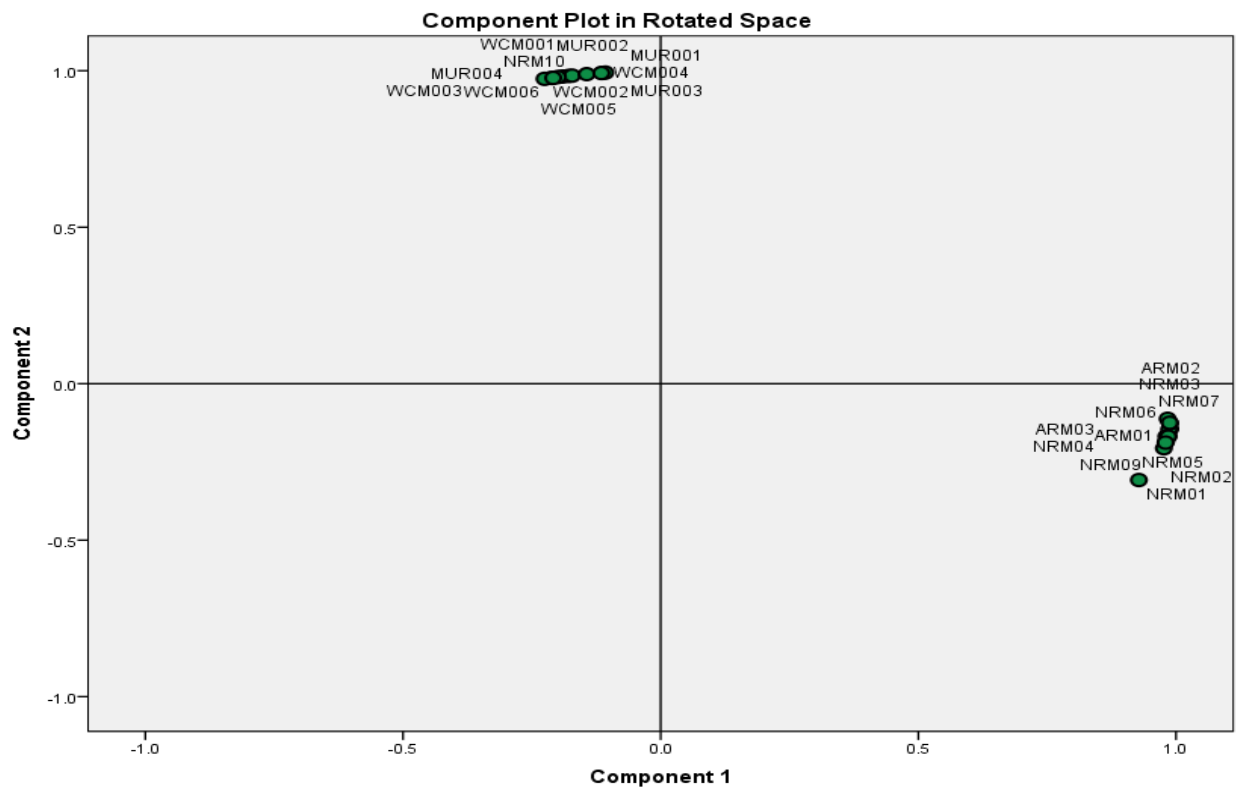


Fig 6 PCA plot of the 23 rice accessions based on standardized grain morphological traits

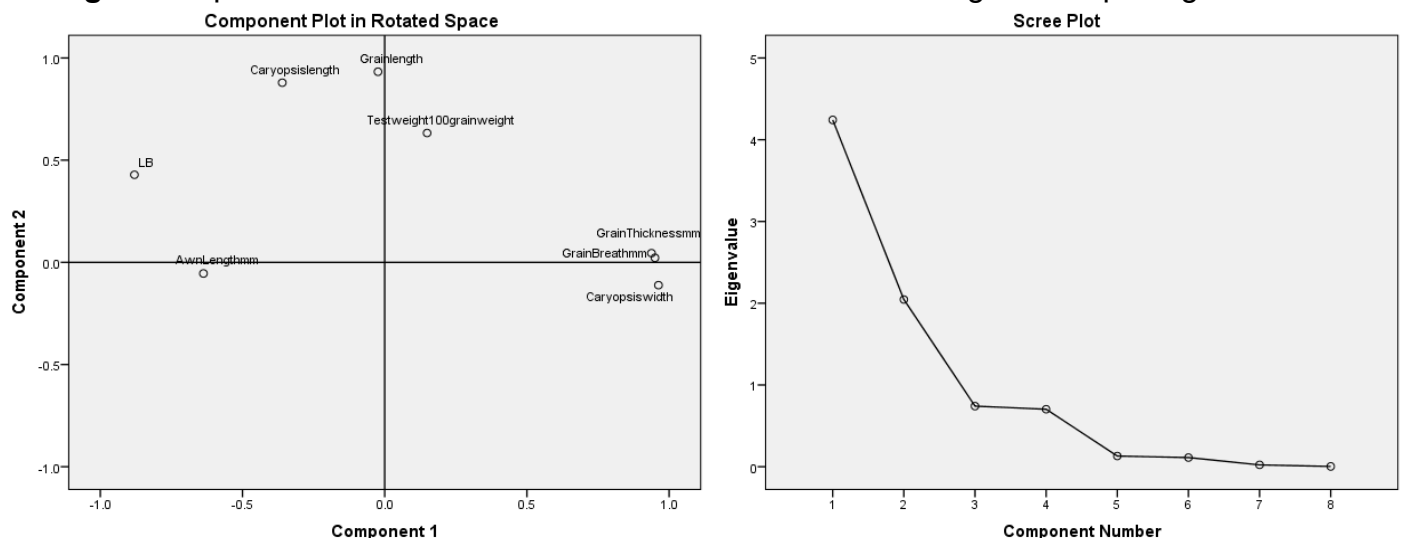


Fig 6 Principal component analysis of eight grain morphological traits and Scree plot based Eigen Values

Table 8. Eigenvalues and percentage of variance explained by principal components based on eight grain morphological traits

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
PC1	4.242	53.024	53.024
PC2	2.045	25.568	78.592
PC3	.741	9.265	87.857
PC4	.703	8.790	96.647
PC5	.131	1.635	98.283
PC6	.112	1.395	99.678
PC7	.023	.281	99.959
PC8	.003	.041	100.000

Hierarchical Cluster Analysis

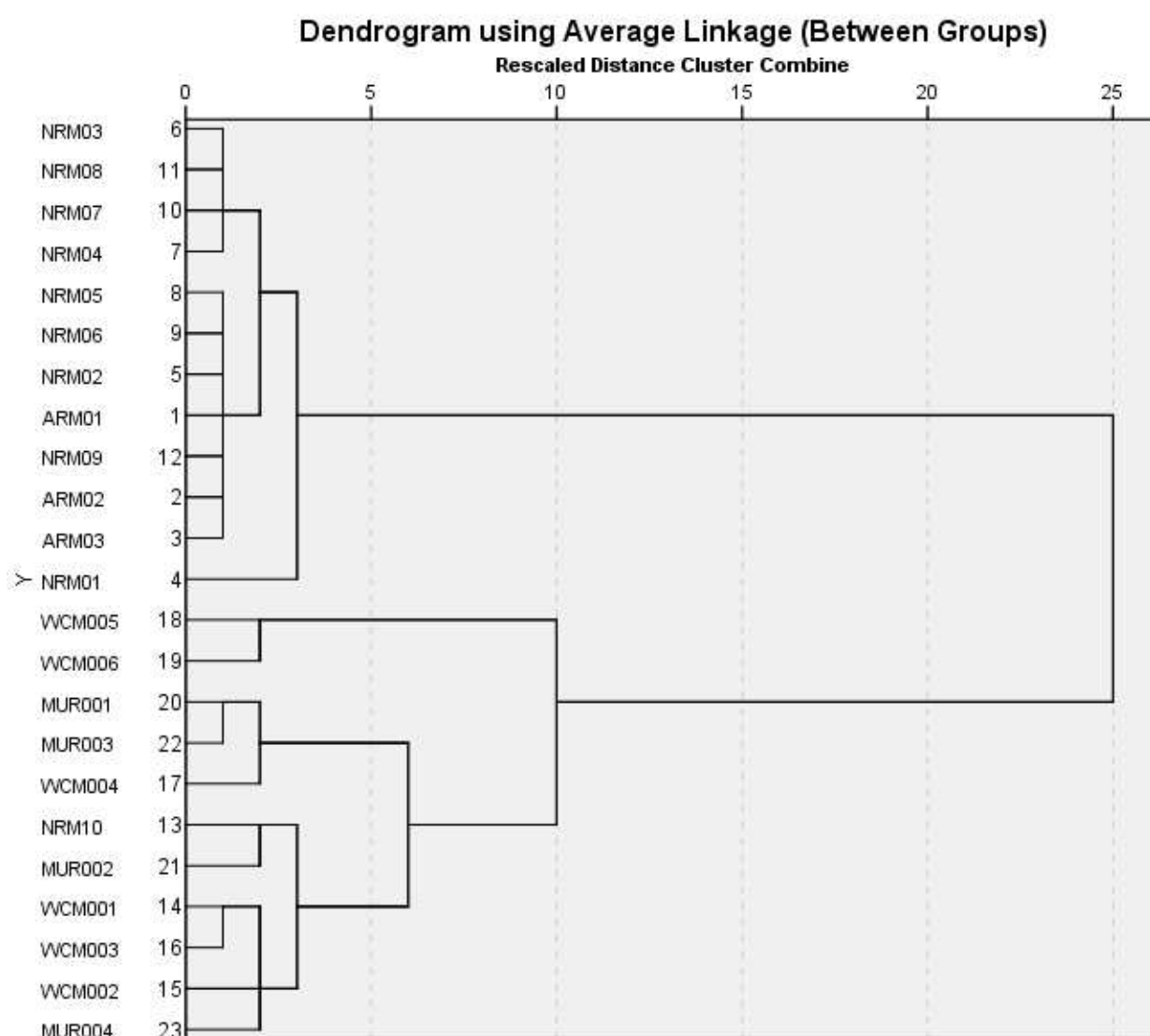


Fig 7. Hierarchical cluster dendrogram (Euclidean distance with average linkage/UPGMA) constructed using the grain physical characteristics of the 23 rice accessions

Hierarchical cluster analysis using the average linkage (between-groups) method based on grain morphological characters separated the 23 rice accessions into two distinct major clusters at a rescaled distance of approximately 25 (Figure 7). The first major cluster comprises 12 accessions, namely Kumbi Phou (NRM03), Phouren amubi (NRM08), Phouren (NRM07), Lang Phou (NRM04), Phoudum (NRM05), Phougak (NRM06), Kakcheng phou (NRM02), Chakhao amubi (ARM01), Sangsangba (NRM09), Langphou Chakhao (ARM02), Chakhao khongnembi (ARM03) and Heimang Phou (NRM01). This cluster is mostly composed of cultivated rice accessions and exhibited relatively short linkage distances among its members, indicating comparatively high similarity in grain morphological characteristics. Kumbi Phou and Phouren amubi forms the closest pair, joining at approximately 1, followed by the association of Phouren and Lang Phou at approximately 2. Phoudum, Phougak and Kakcheng Phou were subsequently incorporated into this subgroup. Among the aromatic accessions, Langphou Chakhao and Chakhao khongnembi form a close pair at approximately 1, while Chakhao amubi joined the cultivated group at approximately 2–3. Sangsangba was associated with the aromatic subgroup, and Heimang Phou joined the major cultivated cluster at approximately 3–4. The second major cluster comprised 11 accessions, including all six Wainuchara (*O. rufipogon*) accessions (WCM001–WCM006), all four Murshi (*O. nivara*) accessions (MUR001–MUR004), and the non-aromatic accession Taothabi (NRM10). Accessions of Wainuchara from Loktak Paat and ex-situ (WCM005 and WCM006) formed a close pair at approximately 2, whereas accessions of Murshi from Ikop Paat and Rice Research Station, Imphal (MUR001 and MUR003) joined at approximately 1. Wainuchara accessions collected from Waithou Paat (WCM004) subsequently joined the Murshi accessions collected from Ikop Paata and Nganglou Paat (MUR001–MUR003) subgroup at approximately 3. The accessions of Wainuchara from Loktak Paat and ex-situ (WCM005 and WCM006) and the Murshi accessions collected from Ikop Paata, Nganglou Paat and ex-situ (MUR001, MUR003 & WCM004) subgroups merged at approximately 10, indicating greater internal differentiation among these groups. Another subgroup was formed by Wainuchara accessions like WCM001, WCM003 and WCM002, with Murshi (MUR002) subsequently joining this group at approximately 3–4 and Murshi (MUR004) at approximately 4. Taothabi (NRM10) was positioned within this predominantly wild-rice cluster and joined at approximately 4–5.

The dendrogram therefore revealed a clear separation between most cultivated rice accessions and the wild rice accessions. The placement of all Wainuchara and Murshi accessions within the second major cluster indicates that *O. rufipogon* and *O. nivara* shared a relatively distinct grain morphological profile compared with most cultivated accessions. The occurrence of Taothabi within the wild-rice cluster suggests that this accession possessed grain characteristics more similar to the wild-rice group than to the other cultivated accessions. Conversely, the clustering of the aromatic accessions ARM01, ARM02 and ARM03 within the predominantly cultivated cluster indicates greater similarity of their measured grain characteristics to the non-aromatic cultivated accessions than to the wild rice. Therefore, the large separation between the two major clusters at approximately 25 and the relatively smaller within-cluster linkage distances demonstrate substantial differentiation in grain morphological characteristics among the evaluated rice accessions.

DISCUSSIONS

Grain morphology is one of the most important determinants of rice quality because it directly influences milling recovery, consumer preference, market price, and breeding value. The observed variation in 100-grain weight, grain length, grain width, and length-to-breadth (L/B) ratio indicates the existence of high phenotypic diversity within the evaluated germplasm. The average economic sink size of a plant is indicated by its test weight or grain weight. Thus, the greater the test weight, the greater the photosynthetic storage capacity, which in turn enhances grain output (Zhou *et al.*, 2017 & Zhang *et al.*, 2022). The 23 rice accessions exhibited substantial grain-morphological variation, with test weight ranging from 1.552 g (Chakhao Khongnembi) to 3.011 g (Lang Phou), grain length from 6.06 mm (Heimang Phou) to 9.83 mm (Lang Phou), grain width from 2.10 mm (WCM002, Wainuchara from Ikop Paat) to 3.60 mm (MUR002, Murshi from RRS, Imphal), and grain thickness from 1.50 mm (WCM003, Wainuchara from Ikop Paat) to 2.36 mm (Lang Phou), with CVs of 13.07%, 9.28%, 14.80% and 13.10%, respectively, indicating considerable diversity for grain size and shape. Grain length, width and length-to-breadth ratio are important components of rice grain quality and market preference, and their variation is frequently exploited in rice improvement programmes (Bioversity International, IRRI, & WARDA, 2007; FAO, 2004). The L/B ratio showed the greatest variability among the major grain-dimension traits (CV = 17.54%), ranging from 1.91 in Heimang Phou to 4.00 in Wainuchara accessions collected from Ikop Paat. This broad range reflects marked differences from bold to slender grain types. Natural variation for grain length, width and grain aspect ratio, with grain shape being a genetically complex but highly useful component of rice diversity (Wang *et al.*, 2025). Grain length, width and length-to-width ratio are important determinants of rice grain quality and are controlled by multiple genetic loci (Bioversity International, IRRI, & WARDA, 2007; Wei *et al.*, 2016; Wang *et al.*, 2025). Li *et al.*, (2018); Zuo and Li, (2014) also reports that grain size traits are under the combined influence of multiple quantitative trait loci (QTL) and developmental genes that regulate cell division and cell expansion during endosperm development. The analysis revealed a clear predominance of Very Long–Medium grains (12 accessions; 52.17%), followed by Very Long–Slender grains (10 accessions; 43.48%), with no accession in the Long–Medium class. Thus, 22 of 23 accessions (95.65%) were either Very Long–Medium or Very Long–Slender. The Very Long–Medium group comprised Chakhao Amubi, Chakhao Khongnembi, Kakcheng Phou, Lang Phou, Phoudum, Phougak, Sangsangba, Taothabi and all four Murshi accessions, whereas the Very Long–Slender group included Langphou Chakhao, Kumbi Phou, Phouren, Phouren Amubi and all six Wainuchara accessions. This concentration of long and very-long grain types is particularly relevant for germplasm utilization because grain length and L/B ratio are major descriptors of rice grain quality and consumer preference. The Standard Evaluation System recognizes grain length classes and L/B-based grain shape classes, with grain length above 7.5 mm categorized as extra-long and an L/B ratio above 3.0 regarded as slender (IRRI, 2013). The predominance of long/slender phenotypes in the present collection therefore indicates that the germplasm contains potentially valuable donors for improving grain appearance and market-preferred grain types. Grain shape is also associated with cooking and eating characteristics and contributes substantially to the commercial classification of rice (Hori & Sun, 2022). Caryopsis length ranged from 4.40 mm in Heimang Phou to 7.30 mm in Phouren, while caryopsis width varied from 1.80 mm in wild rice accessions WCM001 and WCM005 to 2.91 mm in Heimang Phou. Their CV values (10.77% and 15.75%, respectively) indicated moderate variability. Awn length showed the greatest variation, ranging from 0 to 52.0 mm (mean 18.35 mm; CV

110.33%). All wild rice accessions and the non-aromatic accession Taothabi possessed long awns, whereas the remaining aromatic and non-aromatic accessions were awnless, highlighting awning as a key trait distinguishing wild rice from most cultivated accessions (Liu et al., 2015). Indian *O. rufipogon* and *O. nivara* populations have previously been shown to possess substantial variation in awning and other morphological traits, with long awns being particularly characteristic of wild-rice forms (Singh et al., 2018). The present observation therefore supports the usefulness of awn length as a phenotypic indicator of wild-rice differentiation. Dark-red bran was observed in Chakhao Amubi and Sangsangba, reddish-brown bran in Chakhao Khongnembji, Phougak and Taothabi, red bran consistently characterized the Murshi accessions, while Wainuchara showed predominantly brown or light-brown bran (McClung et al., 2020). Such qualitative variation increases the discriminatory value of grain descriptors for germplasm characterization and provides additional phenotypic information for conservation and utilization. The Bioversity–IRRI–WARDA descriptor system specifically incorporates grain and awn characters for systematic characterization of cultivated and wild *Oryza* germplasm. The predominance of long grain length (>8.0 mm) among all Wainuchara (*O. rufipogon*) and Murshi (*O. nivara*) accessions suggests that the wild rice populations of Manipur possess grain characteristics comparable to several commercially preferred long-grain rice cultivars. Although wild rice species are generally recognized as sources of stress-resistance genes rather than grain-quality traits, the present findings indicate that these wild accessions also have desirable grain morphological characteristics (Bierschenk et al., 2020; Gaikwad et al., 2021). Wild rice populations from South and Southeast Asia, where *O. rufipogon* and *O. nivara* showed extensive variation in grain morphology while maintaining predominantly long and slender grains, reflecting their potential contribution to rice quality improvement (Brozynska et al., 2017; Brar and Khush, 2018).

The accession-wise similarity analysis revealed exceptionally high similarity within the wild-rice groups. The six *O. rufipogon* accessions showed coefficients of 0.9925–0.9999, while the four *O. nivara* accessions showed 0.9943–0.9995. In contrast, cultivated–wild comparisons were predominantly negative (–0.218 to –0.510), demonstrating strong phenotypic differentiation between cultivated and wild groups. High within-species similarity combined with between-group differentiation is frequently observed in wild rice because many grain morphological traits are evolutionarily conserved while adaptive ecological traits exhibit greater variation (Wing et al., 2018). The Pearson correlation analysis further demonstrated that grain dimensions were strongly interrelated. Grain length was positively correlated with grain breadth ($r = 0.860$, $P < 0.01$) and caryopsis length ($r = 0.916$, $P < 0.01$), but negatively correlated with grain thickness ($r = -0.863$, $P < 0.01$). Grain breadth was negatively associated with thickness ($r = -0.796$) and positively with caryopsis length ($r = 0.891$). Particularly strong relationships were observed between grain thickness and L/B ratio ($r = -0.907$) and between L/B ratio and caryopsis width ($r = -0.907$). These relationships indicate that selection for one grain-shape component may indirectly influence other dimensions. Similar strong relationships among grain length, width and aspect ratio have been reported in diverse rice populations, supporting the genetic interdependence of grain-shape traits (Wei et al., 2016; Zhang et al., 2022; Wang et al., 2025). In contrast, test weight showed weak and non-significant correlations with the measured grain dimensions ($r = -0.083$ to 0.369), suggesting that grain mass was comparatively independent of the principal dimensional traits in this collection.

ANOVA identified grain thickness and awn length as the principal traits differentiating aromatic, non-aromatic and wild rice groups. Grain thickness showed a significant group effect ($F = 4.090$, $P = 0.032$), whereas awn length showed a highly significant effect ($F = 42.892$, $P < 0.001$). In contrast, test weight, grain length, grain breadth, L/B ratio, caryopsis length and caryopsis width were not significantly different among the three groups ($P > 0.05$). Tukey's HSD indicated that the difference in grain thickness was specifically between non-aromatic and wild rice, with a mean difference of 0.286 ($P = 0.031$). For awn length, wild rice differed strongly from both aromatic (mean difference = -38.630 , $P < 0.001$) and non-aromatic rice (-35.066 , $P < 0.001$), whereas aromatic and non-aromatic rice did not differ significantly ($P = 0.829$). Thus, awn length appears to be the strongest morphological discriminator between cultivated and wild rice, consistent with previous descriptions of *O. rufipogon* and *O. nivara*, where awning and seed morphology are among the diagnostic characters used for classification (Banaticla-Hilario et al., 2018; Sajib et al., 2018). The absence of significant differences for most grain dimensions suggests that grain size alone may not adequately distinguish the cultivated groups in this relatively small germplasm set.

PCA provided a clear multivariate representation of the observed diversity. PC1 had an eigenvalue of 4.242 and explained 53.024% of the total variation, while PC2 had an eigenvalue of 2.045 and explained 25.568%. Together, the first two PCs accounted for 78.592% of the total variation, while PC1–PC4 accounted for 96.647%. PC1 was primarily associated positively with grain breadth, grain thickness and caryopsis width, and negatively with L/B ratio and awn length. PC2 was mainly associated with grain length, caryopsis length and 100-grain weight. This indicates that the first principal axis primarily represented a contrast between broad/thick grains and slender/awned grains, whereas the second axis reflected variation in grain length and mass. The concentration of most variation in the first two PCs is consistent with recent rice studies showing that a limited number of principal components can effectively summarize grain-size and shape variation (Singh et al., 2018; Islam et al., 2024). Multivariate approaches like ANOVA, similarity coefficients, PCA, etc have been successfully used to characterize morphological variation in wild and cultivated rice germplasm (Singh et al., 2018). The PCA plot separated the accessions into two broad groups. Cluster I comprised all 10 wild-rice accessions together with NRM10 (Taothabi), whereas Cluster II contained the remaining cultivated accessions, including ARM01 to ARM03 and NRM01 to NRM09. This pattern demonstrates that the multivariate grain phenotype largely reflected the cultivated-versus-wild distinction, while the position of Taothabi suggests an unusual morphological affinity with the wild-rice group.

Hierarchical cluster analysis reinforced the PCA pattern by separating the 23 accessions into two major clusters at a rescaled distance of approximately 25. Major Cluster I contained 12 predominantly cultivated accessions, whereas Major Cluster II contained 11 accessions comprising all six Wainuchara (*O. rufipogon*), all four Murshi (*O. nivara*) and Taothabi (NRM10). Within Cluster I, Kumbi Phou and Phouren Amubi formed the closest pair at approximately distance 1, followed by the close association of Phouren and Lang Phou. Among aromatic rice, Langphou Chakhao and Chakhao Khongnembi formed a close pair, whereas Chakhao Amubi subsequently joined the cultivated group. These short linkage distances indicate relatively similar grain phenotypes among these cultivated accessions. Cluster II was characterized by close relationships among wild-rice accessions. The subsequent merging of Wainuchara and Murshi subgroups at higher linkage distances indicates differentiation within the wild-rice materials despite their overall separation from cultivated rice. The agreement between PCA and hierarchical clustering strengthens the

interpretation that the major source of morphological differentiation was the cultivated–wild contrast. Variations among cultivated and wild rice according to grain morphological traits and multivariate analyses confirms the usefulness of PCA for evaluating rice germplasm diversity and identifying genetically divergent breeding materials (Zhang *et al.*, 2022; Singh *et al.*, 2024). The close association between several *O. rufipogon* and *O. nivara* accessions also reflects their shared AA genome and evolutionary relationship despite their ecological differences, consistent with previous molecular and morphological investigations (Stein *et al.*, 2018; Zhang *et al.*, 2023). The occurrence of Taothabi within the wild-rice cluster is therefore particularly noteworthy and may indicate either retention of ancestral grain characteristics or convergence toward a wild-rice-like phenotype. Thus, the results demonstrate substantial grain morphological diversity among the 23 Manipur rice accessions, with particularly strong differentiation in awn length, grain shape, grain thickness and caryopsis dimensions. The predominance of Very Long–Medium (52.17%) and Very Long–Slender (43.48%) grains, together with the strong separation of cultivated and wild materials in PCA and hierarchical clustering, indicates considerable potential for utilizing these accessions as sources of grain-quality and morphological diversity (Sengupta *et al.*, 2015; Bhuvaneswari *et al.*, 2020). The wild accessions, particularly Wainuchara and Murshi, provide distinctive phenotypes such as very long slender grains and exceptionally long awns, whereas cultivated aromatic accessions contribute diverse grain and bran characteristics. Because grain length, width and L/B ratio are important targets in rice breeding and are known to have a genetic basis involving multiple loci, these phenotypically divergent accessions could provide useful parental material for future breeding and marker-trait association studies (Wei *et al.*, 2016; Wang *et al.*, 2025). The unique grain characteristics identified in the Manipur wild rice germplasm further emphasize the importance of this region as a valuable centre of rice genetic diversity. These accessions constitute promising genetic resources for broadening the cultivated rice gene pool and for developing future varieties combining superior grain quality with enhanced adaptation to biotic and abiotic stresses (Sengupta *et al.*, 2015; Zhang *et al.*, 2022).

CONCLUSIONS

The present study demonstrated substantial grain morphological diversity among the 23 rice accessions from Manipur, with pronounced variation in grain size, shape, caryopsis dimensions, bran colour and awn length. The predominance of Very Long–Medium (52.17%) and Very Long–Slender (43.48%) grain types highlight the potential of the evaluated germplasm for improving market-preferred grain characteristics. Awn length emerged as the strongest discriminator between cultivated and wild rice, with a highly significant group effect ($F = 42.892$, $P < 0.001$), while grain thickness also showed significant differentiation ($F = 4.090$, $P = 0.032$). Multivariate analyses further confirmed clear differentiation between cultivated and wild rice. The first two principal components explained 78.592% of the total variation, while PCA and hierarchical clustering consistently grouped the wild *O. rufipogon* and *O. nivara* accessions separately from most cultivated accessions. Notably, Taothabi showed morphological affinity with the wild-rice group, warranting further investigation. These findings demonstrate that the indigenous rice germplasm of Manipur represents a valuable reservoir of grain-quality and morphological diversity. In particular, Wainuchara and Murshi accessions possessing very long, slender grains and distinctive awning characteristics may serve as promising donor materials for pre-breeding and rice improvement. Their incorporation into breeding programmes could contribute to broadening

the cultivated rice gene pool and developing varieties that combine desirable grain quality with adaptation to biotic and abiotic stresses. The identified phenotypic diversity also provides a useful foundation for future molecular characterization, marker–trait association studies and targeted conservation of Manipur’s rice genetic resources.

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Syed S H conceptualized designed the article, data collection, designed the methodology, wrote the manuscript, and approved the final version.

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DECLARATIONS

Consent for publication

Not applicable. The present study did not involve human participants or human subjects. Therefore, consent to publish was not required.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethics

The plant materials examined in this study comprised aromatic, non-aromatic, and wild rice accessions, including the wild rice types Wainuchara (*Oryza rufipogon* Griff.) and Murshi (*Oryza nivara* Sharma et Shastry). The accessions were obtained from the Rice Research Station, Imphal, Manipur, as well as collected from swampy habitats and shallow lakeshore regions surrounding Ikop Paat, Waithou Paat, and Loktak Paat in Thoubal and Bishnupur districts of Manipur, India. The local names assigned to the rice accessions were compiled in accordance with locally recognized and nationally applicable guidelines and nomenclature practices, as documented by the Department of Agriculture and the Office of the Rice Breeder, Rice Research Station, Khangabok, Imphal, Manipur. The wild rice collection sites are situated on government-owned land and are traditionally accessible to local communities and researchers; therefore, no specific collection permits, permissions, or licences were required for the present study. Furthermore, the wild rice taxa examined in this study are not listed among the endangered or legally protected plant species of Manipur. The collection, handling, conservation, and utilization of the plant materials were conducted in accordance with applicable institutional, local, and national guidelines governing the collection and use of plant genetic resources, including the Biological Diversity Act and relevant associated regulations, wherever applicable. All accessions were collected exclusively for scientific research, morphological characterization, and germplasm conservation purposes. The materials were maintained and utilized solely for research and were not subjected to commercial exploitation. Appropriate care was taken throughout the collection, handling, maintenance, and utilization of the germplasm to ensure compliance with applicable governmental and institutional requirements.

Consent to participate

Not applicable.

Plant Ethics

A. Plant identification: The cultivated aromatic and non-aromatic rice were identified and issued by Department of Agriculture, Office of the Rice Breeder, Rice Research Station, Khangabok, Imphal, Manipur under receipt no-0042/2024. The wild rice accessions were identified based on their morphological characteristics and relevant taxonomic/descriptive standards. The plant materials were identified by Medhabati Kangabam, Assistant Professor, Department of Botany, Dhanamanjuri University, based on relevant taxonomic and morphological characteristics.

B. Herbarium details: Voucher specimens of the wild rice materials were deposited in Herbarium storage room of Department of Botany, Dhanamanjuri University and identified by Medhabati Kangabam, Assistant Professor, Department of Botany, Dhanamanjuri University under voucher/deposition numbers (not provided yet).

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Supplementary Files

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