Research Paper

Morphological and Molecular Characterization of *Ex-situ* Conserved Kaluheenati (*Oryza sativa* L.) Traditional Rice Accessions in Sri Lanka

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Abstract

This research focused on characterizing 30 Kaluheenati rice accessions, conserved at the Plant Genetic Resources Centre (PGRC), Sri Lanka, using morphological traits and molecular markers. The study aimed to assess the extent of diversity within the Kaluheenati collection, to identify accessions suitable for quality seed production, and identify duplications in the PGRC seed collection. The trial was conducted in 2022 *Yala* and 2022/23 *Maha* seasons at the PGRC. The study recorded 44 morphological traits and used 30 Simple Sequence Repeat markers, and significant morphological diversity was observed in the tested traits except for variety group, ligule shape, collar color, flag leaf angle, lemma and palea pubescence and panicle threshability. Principal component analysis and cluster analysis grouped the accessions into seven clusters highlighting the variations. SSR analysis detected 147 alleles, indicating a diverse gene pool. Polymorphic Information Content ranged from 0.31 to 0.75, indicating moderate to high levels of polymorphism. The phylogenetic tree revealed five distinct clusters, indicating genetic variations within the Kaluheenati accessions. Ten accessions (AC 016955, AC 016944, AC 012926, AC 004091, AC 006713, AC 004089, AC 004087, AC 005191, AC 005385, and AC 007802), including pure lines from the RRDI and conserved accession at International Rice Research Institute clustered together, demonstrating genetic similarities. Despite name similarities, no exact genetic duplications were observed. These findings emphasize the genetic diversity present within the conserved Kaluheenati accessions, highlighting the importance of correct identification and utilization for future breeding programs.

Keywords: **Genetic diversity, Germplasm conservation, Morphological traits, Sri Lanka, SSR markers, Traditional rice**

Introduction

Rice is of immense importance as a staple food for over half of the world's population, providing a significant source of calories, nutrients, and livelihoods (Mohidem, *et al*., 2022). Rice varieties cultivated in Sri Lanka from ancient times until the green revolution are referred to as traditional rice. Those traditional rice varieties have been conserved, developed and used by Sri Lankan farmers for over 3000 years and they passed down from preceding generations (Rambukwella and Priyanka, 2016). These particular varieties exhibit resilience towards both biotic and abiotic stresses and, play a significant role in preserving the biodiversity of Sri Lanka (Weerakoon and Somaratne, 2021). The most commonly cultivated traditional rice varieties in Sri Lanka are Suwadel, Kaluheenati, Kuruluthuda, Pachchaperumal, Suduru samba, Madathawalu and Pokkali (Wasala *et al.,* 2017). A global perspective of the richness of traditional crop variety diversity is maintained by farming communities. Further to that, substantial crop diversity is being conserved in gene banks all over the world. Currently, the PGRC, Sri Lanka conserves about 16,882 accessions of crop germplasm, out of which 5773 are rice and related species. Among the rice accessions, 2497 are traditional rice accessions under 893 different cultivar names (PGRC information). Accurate identification and production of high-quality seeds are essential due to increased demand for cultivation of traditional rice and the presence of numerous accessions sharing the same cultivar name.

Kaluheenati, a traditionally cultivated red rice variety, has gained popularity among consumers due to its higher nutritive value, and antioxidant content (Abeysekera *et al.,* 2017). The nutritional composition of Kaluheenati was determined to be 76% carbohydrates, 11% crude protein, 2.6% crude fat, and 0.9% fiber (Kariyawasam *et al.,* 2016). Herath *et al.* (2011) reported that Kaluheenati exhibited a notable iron content of 4.2 ppm in the endosperm with 8.7% bioavailable iron. Moreover, Abeysekera *et al.* (2017) suggested that regular consumption of Kaluheenati may be useful in dietary management and the prevention of age-related diseases, such as diabetes, cancer, cardiovascular, and neurological diseases, and attributed this feature to its higher levels of naturally derived antioxidants.

A total of 58 rice accessions, known as Kaluheenati have been collected from various regions of Sri Lanka and are currently conserved at the PGRC gene bank. Ensuring the identification of duplicate accessions is essential for maintaining accuracy, resource efficiency, and the integrity of valuable genetic materials in gene bank. Siriwardhana *et al.* (2016) characterized 35 Kaluheenati accessions using SSR markers and identified nine accessions closely related to its pure line (AC 721 of RRDI rice collection). However, several uncharacterized Kaluheenati accessions remain in the PGRC gene bank. Hence, the primary objective of this study was to comprehensively characterize the remaining non-characterized Kaluheenati accessions at the PGRC gene bank, in

conjunction with previously identified accessions, using morphological characters and SSR molecular markers. The aim was to elucidate the extent of diversity within the Kaluheenati collection and to identify accessions that share identical characteristics, which would be suitable for quality seed production and promotion among farmers and to avoid resource duplications in the PGRC seed collection.

Materials and Methods

Morphological characterization

The study included 30 Kaluheeanati rice accessions, with 28 obtained from the PGRC seed gene bank and two pure lines (AC 016944, AC 016955) sourced from the RRDI (Table 1). Improved rice variety, Bg 360 was used for the comparison of morphological data with the traditional rice used. The experiments were carried out at the PGRC, Gannoruwa from 2022 to 2023. The average data for the 2022 *Yala* and *Maha* seasons were used to analyze the morphological characters.

Seeds were germinated on seed trays, and after 2 weeks, three uniform and healthy seedlings were transplanted in distinct pots containing mud soil as a potting medium. The experiment was carried out in the plant house of the PGRC, and pots were arranged in a Complete Randomized Design with three replicates during the 2022 *Yala* and 2022/23 *Maha* seasons. In accordance with the standard descriptor for rice (PGRC, 1999), a total of 30 qualitative and 14 quantitative morphological data were documented during the vegetative, flowering and maturity stages. The recorded data included 8 culm characters, 12 leaf characters, 8 floral characters, 7 panicle characters, 6 grain characters, and 3 growth characters (Table 2).

Table 1. Kaluheenati accessions used for the study

Accession No.	Collected area	Accession No.	Collected area
AC 002089	Gannoruwa	AC 012926	Monaragala
AC 002100	Gannoruwa	AC 013271	Trincomalee
AC 003147	Unknown	AC 013635	Matale
AC 003200	Unknown	AC 013709	Kurunegala
AC 003471	Unknown	AC 014035	Nuwaraeliya
AC 004087	Unknown	AC 014609	Kegalle
AC 004089	Unknown	AC 014611	Kurunegala
AC 004091	Unknown	AC 014673	Kegalle
AC 005191	Badulla	AC 015061	Unknown
AC 005385	Ambalanthota	AC 015213	Gampaha
AC 005484	Kurunegala	AC 015216	Gampaha
AC 006713	IRRI conserved Acc	AC 015377	Anuradhapura
AC 007802	Kandy	AC 015638	Kandy
AC 010728	Anuradhapura	AC 016944	RRDI (pure line)
AC 011089	Kegalle	AC 016955	RRDI (AC 721)

Table 2. Morphological data recorded in the study

Data category	Qualitative characters	Quantitative characters
Culm /Stem	Culm angle after flowering, Internode color after flowering, Culm strength, Collar color at late vegetative stage	Seedling height (cm), Culm diameter at flowering (cm), Culm number after full heading, Culm length (cm)
Leaf	Leaf blade pubescence, Leaf blade color, Basal leaf sheath color, Leaf angle, Ligule shape, Ligule color, Auricle color, Flag leaf angle, Leaf senescence	Ligule length (mm), Leaf blade width (cm), Leaf blade length (cm)
Floral	Stigma color, Lemma & palea pubescence at flowering, Sterile lemma color, Lemma and palea color, Spikelet sterility, Apiculus color, Anther color	Sterile lemma length (mm)
Panicle	Panicle type at near maturity, Secondary branching, Panicle exertion, Panicle axis at maturity, Panicle shattering, Panicle threshability	Panicle length (cm)
Grain	Seed coat (bran) color, Awning after full heading, Awn color	Grain length (mm), Grain width (mm), 100 grain weight (g)
Growth habit	Variety group	Days to maturity, Days to heading

Molecular characterization

Genomic DNA was extracted from two weeks old immature tender leaves using the CTAB method (Doyle and Doyle, 1990) with slight modifications. Thirty SSR markers similar to those used by Siriwardhana *et al*. (2016) were used for the genotyping. The PCR amplification followed the protocol described by Wasala *et al*. (2012). The PCR products were confirmed on a 1.5% Agarose gel and separated using 8% non-denaturing polyacrylamide gel electrophoresis. Bands were visualized by the gel documentation system (BIO RAD) after staining with Ethidium Bromide.

Data analysis

Morphological data analysis

Quantitative data analysis was performed using SAS 9.00 (SAS Institute Inc., USA, 2021) employing analysis of variance and Duncan Multiple Range Test (DMRT) for mean comparisons. MINITAB var 17 was utilized for cluster analysis and Principal component analysis (PCA) to examine morphological diversity among the Kaluheenati accessions based on qualitative and quantitative data. Variety type, panicle type, auricle color, flag leaf angle, ligule shape and collar color were not considered for PCA since the data for all accessions were similar.

Molecular data analysis

The banding pattern of the SSR marker was recorded using Quantity one 4.6 software. Cluster analysis was conducted using the Unweighted Paired Group Method with Arithmetic Average (UPGMA) algorithm and Nei's genetic distance (Nei and Chesser, 1983), with the assistance of POWER MARKER V 3.25 software (Liu and Muse, 2005). A phylogenetic tree was constructed by combining MEGA 11 and POWER MARKER V 3.25, using the scored data.

Results and Discussion

Morphological characterization

Data of morphological traits in 2022 *Yala* and 2022/23 *Maha* seasons were analyzed across seasons and, a majority of the traits exhibited similarity. Notable variations were observed in several qualitative characters, highlighting the genetic variability within the Kaluheenati accessions studied.

Quantitative traits

Accessions in the study exhibited significant differences for quantitative traits measured (Table 3). Culm length (CL) ranged from 57 cm to 117 cm, with accessions showing significant differences. Eight accessions namely AC 013635, AC 002100, AC 003200, AC 013709, AC 007802, AC 004089, AC 005191 and AC 016955 were above 100 cm in CL. All these accessions were clustered together in Cluster 3 except for AC 013635. Hence, Culm length could be used as a morphological trait to group similar accessions. Culm diameter (CD) ranged from 1.07 to 1.6 cm. Leaf blade length (LBL) reached the highest at 71.1 cm in accession AC 003200, while leaf blade width varied from 0.8 to 1.4 mm. Ligule lengths (LL), ranged from 1.2 cm to 3.10 cm.

Panicle length showed significant variation from 21 cm to 30 cm. Grain length varied from 6 to 9.2 mm, and width ranged from 2.4 to 3.5 mm. The highest 100 grain weight (GW) was recorded by AC 003471 (3.4 g), while the lowest recorded by AC 013271 (1.5 g). Days to heading and Days to maturity exhibited variations among the accessions, indicating differences in their growth and development patterns.

Qualitative characters

All the rice accessions were uniform for five of the qualitative characters among 30 studied. It includes variety group (Indica), ligule shape (2-cleft), collar color at the late vegetative stage (pale green), flag leaf angle (erect) and panicle threshability (intermediate). Lemma and palea pubescence of all Kaluheenati accessions were recorded as short hairs while Bg 360 recorded it as long hairs (data not shown). Three different culm angles after flowering were observed, with 9, 20 and 2 accessions showing an erect angle, an intermediate angle (45^0) and an open angle (60^0), respectively. Except for six accessions which recorded internode color as green, all the other accessions showed light gold color internodes. Culm strength, which is also known as the lodging resistance, varied with five accessions showing strong culm strength, while 23 accessions as moderately strong and one accession (AC 016944) as weak. Four accessions (AC 002089, AC 002100, AC 005484 and AC 015377) showed glabrous pubescence, while 16 accessions gave intermediate pubescence in leaf blade and 9 accessions recorded pubescent. Leaf blade color at late vegetative stage was diverse with four colors of pale green (18 accessions), green (9 accessions), dark green (2 accessions) and purple tips (AC 012926 and Bg 360). Basal leaf sheath color also varied as green (18 accessions), purple lines (9 accessions), light purple (2 accessions- AC 003147 and AC 013635) and purple (AC 012926 and Bg 360). White ligule color was observed in 17 accessions while purple lines were observed in the rest of the accessions.

Table 3. Quantitative morphological traits of Kaluheenati rice accessions

Accession No.	SH	LBL	LBW	LL	CL	CD	PL
AC 002089	52.89 ^{jkl}	47.50^{ijkl}	1.16^{cdef}	2.33^{cde}	57.11^{m}	1.07^{o}	24.56^{ghi}
AC 002100	55.33^{hijkl}	56.67^{defgh}	1.16^{cdef}	1.92^{fghi}	107.89^{bc}	1.63^{a}	28.78^{bc}
AC 003147	71.44^{ab}	52.56^{fghij}	0.89^{l}	1.23^{m}	99.44^{defgh}	1.34^{fghijk}	27.33^{cd}
AC 003200	71.56^{ab}	71.11^{a}	1.16^{cdef}	3.10^{a}	102.11^{cdef}	1.33^{fghijkl}	30.56^{a}
AC 003471	70.667^{abc}	57.19^{cdefg}	1.18^{bcdef}	1.98^{efgh}	79.33^{l}	1.51^{bcd}	28.94^{bc}
AC 005484	44.89^{m}	33.69^{mn}	1.19^{bcde}	1.48^{klm}	61.89^{m}	1.37^{efghij}	24.33^{ghi}
AC 010728	68.33^{abcd}	65.56^{abc}	1.30^{ab}	3.07^{a}	115.78^{a}	1.43^{cdefg}	24.78^{fgh}
AC 011089	62.33^{defghi}	56.89^{defg}	1.06^{fghij}	2.43^{cd}	99.56^{defgh}	1.40^{defghi}	29.06^{b}
AC 013271	53.22^{jkl}	59.00^{bcdef}	0.99^{hijkl}	1.90^{ghij}	93.11^{ghi}	1.23^{klmn}	26.33^{def}
AC 013635	73.56^{a}	55.22^{efghi}	0.92^{kl}	1.40^{lm}	103.44^{cde}	1.49^{bcde}	26.00^{defg}
AC 013709	52.33$^{k\ lm}$	65.78^{ab}	1.09^{efgh}	2.10^{defg}	100.22^{cdefg}	1.42^{cdefgh}	27.60^{1bcd}
AC 014035	62.22^{defghi}	49.07^{ghijkl}	0.98^{hijkl}	1.52^{ijklm}	87.44^{ijk}	1.27^{jklm}	22.94^{ij}
AC 014609	58.56$^{f\ hijkl}$	49.17^{ghijkl}	1.06^{fghij}	1.62^{hijklm}	95.67^{efgh}	1.30^{hijklm}	21.89^{j}
AC 014611	61.56^{defghi}	46.17^{ijkl}	0.96^{ijkl}	1.50^{jklm}	95.00^{fghi}	1.21^{lmn}	23.83^{hi}
AC 014673	67.22^{abcde}	47.78^{hijkl}	0.93^{jkl}	1.61^{hijklm}	92.78^{ghi}	1.28^{ijklm}	23.56^{hi}
AC 015061	55.11^{hijkl}	61.98^{bcde}	1.08^{efghi}	1.99^{efgh}	77.22^{l}	1.28^{ijklm}	21.72^{j}
AC 015213	56.22^{ghijkl}	65.02^{abcd}	1.02^{ghijk}	2.53^{c}	97.11^{defgh}	1.13^{no}	28.44^{bc}
AC 015216	54.56^{ijkl}	49.88^{ghijk}	0.96^{ijkl}	1.74^{ghijkl}	92.00^{hij}	1.31^{ghijklm}	23.78^{hi}
AC 015377	64.44^{bcdefg}	54.00^{efghi}	0.98^{hijkl}	1.46^{lm}	84.44^{jkl}	1.22^{klmn}	24.39^{ghi}
AC 015638	64.33^{bcdefg}	53.50^{efghij}	1.01^{ghijkl}	1.5^{jklm}	94.00^{ghi}	1.26^{jklm}	25.06^{efgh}
AC 004089	64.89^{bcdef}	46.56^{ijkl}	1.23^{bcd}	2.92^{ab}	113.78^{ab}	1.53^{abc}	25.22^{efgh}
AC 005191	65.44^{abcdef}	56.67^{defgh}	1.20^{bcde}	2.56^{bc}	104.56^{cd}	1.43^{cdefg}	26.50^{de}
AC 005385	50.44^{ml}	40.33^{ml}	1.40^{a}	2.30^{cdef}	79.67^{l}	1.59^{ab}	26.33^{def}
AC 007802	68.33^{abcd}	60.22^{bcdef}	1.10^{efgh}	2.68^{bc}	117.22^{a}	1.53^{abc}	24.56^{ghi}
AC 004087	67.67^{abcde}	47.11^{ijkl}	1.13^{defg}	2.64^{bc}	78.33^{l}	1.37^{efghij}	28.67^{bc}
AC 006713	52.22^{klm}	50.00^{ghijk}	1.12^{defg}	1.87^{ghijk}	87.33^{ijk}	1.34^{fghijk}	28.67^{bc}
AC 012926	60.78^{defhij}	46.00^{jkl}	1.06^{fghij}	1.72^{ghijkl}	87.67^{ijk}	1.40^{defghi}	21.89^{j}
AC 004091	63.11^{cdefgh}	46.44^{ijkl}	0.93^{jkl}	1.98^{efgh}	99.00^{defgh}	1.20^{mn}	24.56^{ghi}
AC 016955	59.78^{efhijk}	42.60^{kl}	1.20^{bcde}	2.60^{bc}	104.44^{cd}	1.44^{cdef}	26.44^{de}
AC 016944	63.00^{cdefgh}	51.67^{fghij}	1.28^{bc}	1.94^{efgh}	83.56^{kl}	1.46^{cdef}	22.89^{ij}
Bg 360	32.00^{n}	30.20^{n}	1.18^{bcdef}	1.76^{ghijkl}	61.67^{m}	1.33^{fghijkl}	25.22^{efgh}
CV%	12.3	5.3	10.7	8	7.9	8.5	5.9

Within the column the means followed by the same letter are not significantly different at P=0.05
SH - Seedling height (cm), LBL- Leaf blade length (cm), LBW- Leaf Blade width (cm), LL-Ligule length (cm), CL-Culm length (cm), CD - Culm diameter (cm), PL - Panicle length (cm), GL - Grain length (mm), GWI- Grain width (mm), CN- Culm number after full heading, SLL- Sterile lemma length (mm), DTH-Days to heading, DTM- Days to maturity, GW-100 Grain weight (g)

Table 3. Quantitative morphological traits of Kaluheenati rice accessions (continued)

Accession No.	GL	GWI	CN	SLL	DTH	DTM	GW
AC 002089	8.77^{bc}	2.45^{p}	23^{b}	3.00^{a}	92^{m}	129^{h}	2.03^{r}
AC 002100	8.19^{de}	2.91^{jkl}	9^{g}	3.00^{a}	101^{i}	140^{e}	2.30^{l}
AC 003147	9.29^{a}	2.79^{mn}	6^{j}	3.00^{a}	77^{p}	129^{h}	2.80^{d}
AC 003200	8.18^{de}	2.97^{ijk}	6^{j}	3.00^{a}	106^{g}	140^{e}	2.1^{p}
AC 003471	8.92^{b}	3.42^{b}	5^{k}	3.00^{a}	66^{q}	116^{m}	2.90^{c}
AC 005484	9.28^{a}	2.96^{ijk}	20^{c}	3.00^{a}	83^{n}	129^{h}	2.70^{e}
AC 010728	8.05^{def}	3.23^{de}	8^{h}	3.00^{a}	106^{g}	140^{e}	2.60^{f}
AC 011089	8.16^{de}	3.37^{bc}	7^{i}	3.00^{a}	94^{l}	140^{e}	3.00^{b}
AC 013271	6.03^{n}	2.91^{jkl}	10^{f}	3.01^{a}	99^{j}	129^{h}	1.57^{w}
AC 013635	7.85^{efgh}	3.02^{ghi}	6^{j}	3.00^{a}	79^{o}	140^{e}	2.40^{j}
AC 013709	7.09^{m}	2.56^{o}	9^{g}	1.0^{b}	99^{j}	129^{h}	1.67^{v}
AC 014035	8.26^{d}	3.10^{fg}	16^{d}	3.01^{a}	77^{p}	121^{k}	2.40^{j}
AC 014609	7.93^{efgh}	3.11^{fg}	8^{h}	3.00^{a}	77^{p}	129^{h}	2.57^{g}
AC 014611	8.22^{de}	3.11^{fg}	9^{g}	3.00^{a}	77^{p}	129^{h}	2.50^{h}
AC 014673	8.00^{defg}	2.99^{hij}	10^{f}	3.00^{a}	77^{p}	126^{i}	2.47^{i}
AC 015061	7.50^{1ijkl}	2.91^{jkl}	8^{h}	1.01^{b}	77^{p}	129^{h}	2.07^{q}
AC 015213	7.25^{lm}	2.74^{n}	8^{h}	3.00^{a}	96^{k}	141^{d}	2.17^{o}
AC 015216	7.41^{kl}	3.08^{ghi}	7^{i}	3.00^{a}	77^{p}	140^{e}	2.37^{k}
AC 015377	7.52^{hijk}	2.98^{ij}	31^{a}	3.00^{a}	77^{p}	129^{h}	2.27^{m}
AC 015638	8.22^{de}	2.87^{klm}	9^{g}	3.01^{a}	77^{p}	120^{l}	2.50^{h}
AC 004089	7.75^{fghi}	3.16^{ef}	4^{l}	3.00^{a}	125^{a}	147^{b}	2.90^{c}
AC 005191	8.26^{d}	3.56^{a}	4^{l}	3.00^{a}	116^{d}	131^{g}	3.10^{a}
AC 005385	8.17^{de}	2.81^{lmn}	5^{k}	3.00^{a}	125^{a}	145^{c}	2.00^{s}
AC 007802	8.31^{d}	3.22^{de}	4^{l}	3.00^{a}	116^{d}	136^{f}	2.70^{e}
AC 004087	8.61^{c}	3.29^{cd}	5^{k}	3.00^{a}	115^{e}	136^{f}	2.70^{c}
AC 006713	7.66^{ghij}	2.81^{lmn}	5^{k}	3.00^{a}	121^{c}	136^{f}	1.90^{t}
AC 012926	7.03^{m}	3.35^{bc}	4^{l}	3.00^{a}	108^{f}	125^{j}	1.80^{u}
AC 004091	7.81^{fgh}	3.16^{ef}	4^{l}	3.00^{a}	115^{e}	136^{f}	2.00^{s}
AC 016955	7.73^{ghij}	3.11^{fg}	8^{h}	3.00^{a}	123^{b}	165^{a}	2.20^{n}
AC 016944	7.99^{defg}	3.09^{fgh}	13^{e}	3.00^{a}	103^{h}	136^{f}	2.30^{l}
Bg 360	6.10^{n}	2.49^{op}	10^{f}	1.00^{b}	96^{k}	129^{h}	1.27^{x}
CV%	3.6	3.3	9.5	0.6	0.9	0.6	1.2

Leaf characteristics such as pubescence, color, angle, auricle color, and senescence exhibited diversity among accessions.

At the maturity, the panicle type of accessions was either compact or intermediate. Eight accessions (AC 002089, AC 002100, AC003147, AC 003471, AC 005484, AC 015213, AC 005385 and Bg 360) recorded intermediate type panicles at maturity while all the other accessions showed compact type panicles. Panicle types, secondary branch types, panicle exertion, and shattering also varied.

The seed coat colors of the tested accessions were diverse with white, red, brown and light brown colors. Among the tested Kaluheenati accessions, a white colored seed coat was observed in AC 002089, AC 002100, AC 003147, AC 005484 and AC 010728. Speckled brown color was given by AC 014611. Fourteen accessions recorded brown color while 9 were light brown color. Accession number AC 003471 recorded the red seed coat. White color stigma was displayed by 14 accessions while light purple stigma was presented by 11 accessions. White anthers were visible in AC 013271 and AC 015061 while all the other Kaluheenati accessions displayed yellow color anthers. Awn presence and color, stigma color, lemma and palea color, and apiculus color showed variation among accessions.

Principal Component Analysis (PCA)

The PCA of 31 accessions, considering 15 quantitative and 25 qualitative traits, resulted in 19 independent principal components (PCs). The first 7 PCs captured 80 % of the total variance, with PC1 explaining 24% of the variation and being associated with floral, seed and leaf traits. PC2 explained 17.7% of the variance and was influenced by seed and leaf traits. PC3 to PC7 collectively accounted for 38.3% of the variance and were driven by various traits including leaf color, culm strength, grain size and maturity. Figure 1 displays the score plot diagram illustrating the variation of Kaluheenati accessions based on first 2 PC values.

Cluster analysis

The multivariate hierarchical cluster analysis grouped 30 Kaluheenati traditional rice accessions, and improved variety Bg 360 into seven clusters (Figure 2). The dissimilarity between genotypes was calculated using Single Linkage Euclidean Distance and ranged from 9.45 to 28.35. Clusters C2, C5 and C7 contained of single accessions (Bg 360, AC 014673 and AC 003471, respectively). C1 (AC 002089, AC 005484) and C2 (Bg 360) cluster exhibited the lowest culm height, while, AC 002089 and AC 005484 in cluster C1 recorded the highest number of culms after full heading. The majority of accessions (77.42%) were grouped in clusters, C3 and C4. Cluster C3 was further divided into four sub-clusters, with AC 012926, AC 013709, AC 013271, AC 015213, AC 011089, AC 002100 and AC 016944 clustered together indicating high similarity to the pure line obtained from the RRDI. AC 016955 (AC 721 of RRDI rice collection), considered the control for Kaluheenati in previous studies (Siriwardhana *et al.,* 2016), also clustered with cluster C3. This clustering information is valuable for selecting germplasm for future breeding and improvement programs for Kaluheenati rice.

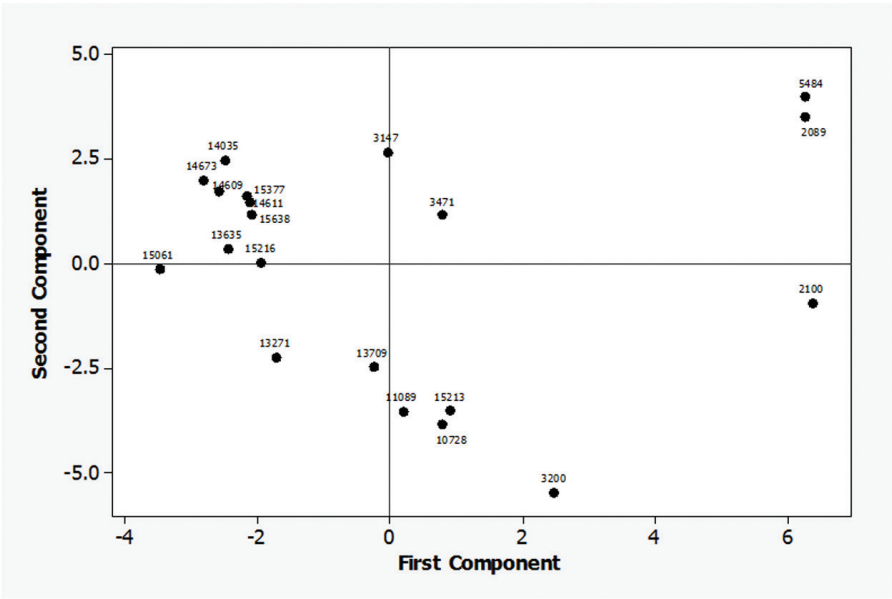

Figure 1. Biplot showing variation of Kaluheenati accessions based on PC1 and PC2 values using 15 quantitative and 25 qualitative traits.

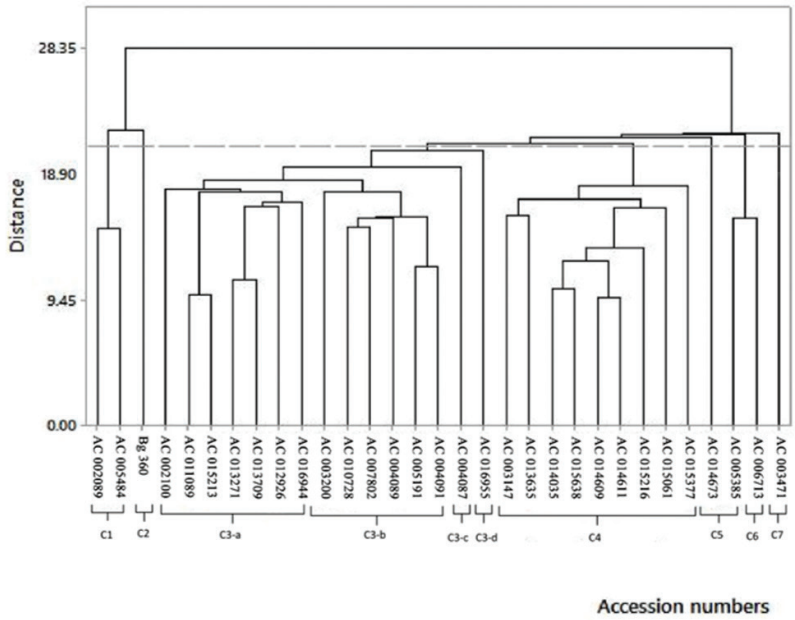

Figure 2. Dendrogram of 30 Kaluheenati rice accessions with Bg 360 improved variety derived by Euclidean Single linkage method of Cluster Analysis

Molecular characterization using SSR markers

Allelic diversity of Kaluheenati germplasm

A total of 147 alleles were detected at 30 polymorphic SSR markers across 30 Kaluheenati traditional rice accessions indicating a diverse gene pool. The average allele richness per marker was 5.74, (Table 4) suggesting a high level of genetic diversity within Kaluheenati rice accessions.

Table 4. Summary of genetic diversity parameters across 30 Kaluheenati accessions with 30 SSR markers

Primer Name	Major Allele Frequency	Genotype No.	Allele Richness	Gene Diversity	Heterozygosity	PIC*
RM20B	0.65	6	4	0.52	0.16	0.47
RM84	0.42	4	4	0.69	0.00	0.63
RM201	0.61	4	4	0.56	0.00	0.50
RM202	0.40	8	5	0.73	0.10	0.69
RM207	0.29	6	6	0.78	0.00	0.75
RM208	0.39	6	5	0.72	0.03	0.67
RM213	0.81	4	3	0.33	0.03	0.30
RM215	0.48	3	3	0.53	0.00	0.42
RM216	0.42	4	4	0.65	0.00	0.59
RM217	0.35	5	5	0.76	0.00	0.73
RM219	0.58	4	4	0.55	0.00	0.48
RM220	0.48	5	4	0.65	0.03	0.59
RM224	0.34	9	8	0.75	0.10	0.70
RM228	0.53	7	7	0.64	0.06	0.60
RM236	0.74	4	3	0.41	0.03	0.37
RM237	0.50	6	4	0.65	0.10	0.60
RM241	0.50	4	4	0.59	0.03	0.51
RM255	0.42	5	6	0.68	0.03	0.62
RM259	0.35	5	5	0.75	0.00	0.71
RM270	0.48	5	5	0.68	0.00	0.63
RM412	0.42	4	5	0.67	0.03	0.61
RM418	0.35	4	4	0.72	0.00	0.67
RM440	0.39	6	7	0.74	0.03	0.71
RM480	0.39	5	4	0.72	0.03	0.67
RM515	0.39	5	5	0.70	0.00	0.65
RM518	0.35	4	4	0.68	0.00	0.62
RM536	0.40	7	7	0.72	0.10	0.67
RM539	0.58	5	6	0.57	0.03	0.51
RM560	0.81	4	5	0.33	0.03	0.31
RM571	0.48	6	7	0.67	0.03	0.62
Mean	0.46	5.97	5.74	0.65	0.03	0.60

**PIC : Polymorphic Information Content*

The average number of genotypes detected for each marker was 5.97, indicating high genetic variation. Major allele frequency ranged from 29 to 81% with an average of 46%, indicating that different markers have varying degrees of allele dominance. This wide range of major allele frequencies is consistent with the presence of a diverse gene pool, as it shows that no single allele is overwhelmingly dominant across all markers, signifying the presence of multiple alleles at significant frequencies. The average gene diversity was 0.65, indicating a high level of genetic diversity. The presence of diversity among the accessions might be due to genetic drift within the population and potential misidentification of cultivars. This was higher than the results of Wasala *et al.* (2017) who studied the genetic diversity among Suwadal rice accessions. The average heterozygosity was as low as 0.03 due to the self-pollinating nature of rice. The Polymorphic Information Content (PIC) refers to the measure of genetic variation or diversity within a population at a specific genetic marker or locus. It quantifies the level of polymorphism, or the presence of multiple alleles, at that marker (Li *et al.,* 2021). A higher PIC value indicates greater diversity and informativeness of the marker in distinguishing among individuals or populations (Anderson *et al.,* 1993). The PIC value ranged from 0.31 to 0.75, suggesting moderate to high levels of polymorphism. RM207 and RM217 markers showed high levels of allele richness, gene diversity, and PIC, making them valuable for assessing genetic diversity in Kaluheenati rice accessions, while RM224 and RM228 markers also exhibited substantial allele richness and gene diversity, indicating their potential for studying genetic structure and differentiation in this population.

Genetic relationship among Kaluheenati accessions

The phylogenetic tree of 30 accessions, revealed five distinct clusters, indicating genetic variations within the Kaluheenati variety (Figure 3). Accessions within each cluster exhibited varying genetic distances, with some accessions showing close relationship. However, no exact genetic duplications were observed, suggesting genetic variations despite similarities among accessions with the same name. The accession, AC 016944 exhibited the lowest genetic distance (0.387) with AC 016955 which was used as a unique Kaluheenati accession in previous studies (Siriwardhana *et al.,* 2016), and clustered closely with the accession from IRRI, Philippines (AC 006713). Nine Kaluheenati accessions, (AC 016944, AC 012926, AC 004091, AC 006713, AC 004089, AC 004087, AC 005191, AC 005385, and AC 007802), showed a close relationship to known Kaluheenati accession, AC 016955, highlighting their potential value in breeding programs. Based on this study and the previous study on Kaluheenati (Siriwardhana *et al.,* 2016), out of all the conserved Kaluheenati accessions (58) in the PGRC, only ten accessions grouped together with the AC 016955, while others showed distinct genetic relationships.

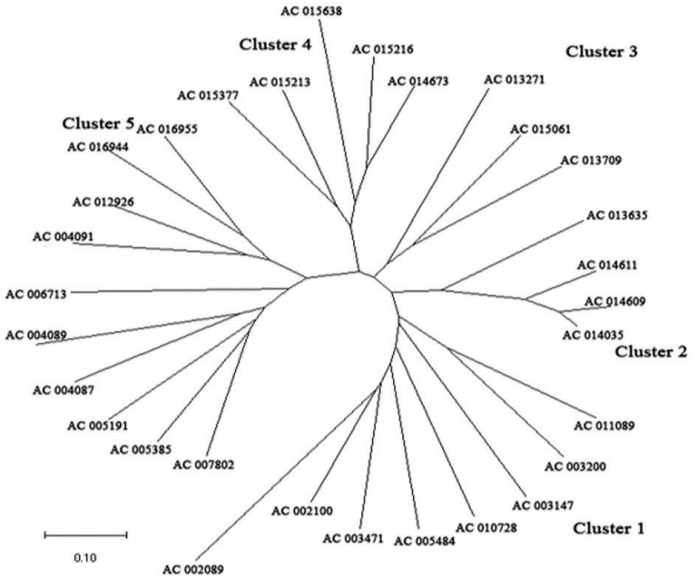

Figure 3. Phylogenetic tree based on Nei's genetic distance (Nei and Chesser, 1983) according to the UPGMA algorithm

Conclusion

In conclusion, the genetic analysis of 30 Kaluheenati rice accessions revealed a substantial amount of genetic variation. No duplicate accessions were found, indicating the uniqueness of each accession. These findings underscore the importance of preserving and utilizing the conserved Kaluheenati accessions for future breeding programs. The presence of significant genetic diversity within the collection provides a valuable resource for developing improved rice varieties with desirable traits, such as higher yield, disease resistance, and adaptability to different environmental conditions. Among the Kaluheenati accessions tested, 9 accessions, (AC 016944, AC 012926, AC 004091, AC 006713, AC 004089, AC 004087, AC 005191, AC 005385, and AC 007802), showed a close relationship to the known Kaluheenati accession (AC 016955) highlighting their potential value in breeding programs. However, it is noteworthy that out of the 58 total conserved accessions, only 10 accessions, exhibited a grouping pattern similar to the known Kaluheenati pure line indicating the genetic diversity within the Kaluheenati collection. Overall, this research contributes valuable insights into the genetic diversity of Kaluheenati rice accessions and highlights the need for continued efforts in their conservation, correct identification, and strategic utilization in breeding programs to ensure food security and agricultural sustainability.

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