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Development of a core collection from Sri Lankan traditional rice (*Oryza sativa*) varieties for phenotypic and genetic diversity

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Abstract. *Weerakoon SR, Somaratne S. 2021. Development of a core collection from Sri Lankan traditional rice (Oryza sativa) varieties for phenotypic and genetic diversity. Nusantara Bioscience 13: 61-67.* A collection of over 2000 traditional rice varieties are conserved at Gene Bank, Plant Genetics Resource Center, Sri Lanka. *Oryza sativa* varieties grown in Sri Lanka from ancient times to the middle of the last century are known as traditional rice. These varieties show adaptability to biotic and abiotic stresses and, an important component of biodiversity of Sri Lanka. A detailed understanding of the diversity of traditional rice varieties is essential for effective utilization of rice genetic resources and identification of potential parents possessing valuable genetic traits for future crop improvement. Study objectives were phenotypic and molecular characterization of one-hundred traditional rice varieties and to identify a core collection for phenotypic and genetic diversity. Rice varieties were grown in a plant house following RCBD with 4 replicates and 5 plants per replicate. Thirty-two agro-morphological characters were observed/collected. Genomic DNA was extracted from 20-days-old seedlings. Thirty-three microsatellite (Simple Sequence Repeat-SSR) primer pairs were used to assay genetic variation and PCR products were subjected to fragment analysis by capillary electrophoresis. Descriptive statistics and basic inferential statistical analyses were performed to access variation of agro-morphological characters among rice varieties. Cluster analysis and Multidimensional scaling produced 07 groups which were further analyzed using Classification and Regression Analysis to extract the diagnostic agro-morphological features. Groups of rice varieties were characterized by lemma palea color, awn color at maturity, seedling height, and flag-leaf angle. Traditional varieties represent distant clusters on agro-morphological features. Molecular analyses revealed all 33 loci displayed polymorphism (66.7-96.9%) among 100 traditional rice varieties with a total of 387 alleles identified with an average of 11.72 alleles per variety. All varieties were genetically structured into fifteen well-separated groups. UPGMA analysis based on Jaccard's similarity separated varieties into 05 major clusters. Genetic diversity information is useful in the efficient use of Sri Lankan rice germplasm and managing *in situ* and *ex situ* germplasm collections in conserving traditional rice varieties.

Keywords: Agro-morphological characterization, core collection, molecular characterization, *Oryza sativa*, traditional rice varieties

INTRODUCTION

Comprehensive knowledge of genetic diversity and population structure of germplasm collections is important for crop improvement. Rice (*Oryza sativa* L.) is the staple food in Sri Lanka, cultivated as a wetland crop in all the districts of the island. According to Department of Agriculture of Sri Lanka reports, rice is the single most important crop occupying 34% percent (0.77 million ha) of the total cultivated area on the island. Rice provides 45% total calories and 40% total protein requirement of an average Sri Lankan (Crop recommendations 2014). Approximately 75% of the rice lands in Sri Lanka are located within the inland valley systems with varying form and size, and the balance of 25% is in coastal plains and associated flood plains (Panabokke 1996). Out of total cultivated amount, around 99% of the area is cultivated with new improved rice varieties. The remaining area is cultivated with traditional rice varieties with low yield. Although new improved varieties produce comparatively higher yields, local and export market demand for traditional rice varieties is higher for their grain qualities, such as high fiber content, despite the lower production (Wickramasinghe and Noda 2008). Moreover, farmer's perceptions (Efisue et al. 2008), improvement of system sustainability (Abeyratne 1956), and the higher adaptability to problem soils (Mandal et al. 1999) further increased interest in traditional rice varieties.

The overall population structure of global rice germplasm has been well characterized. However, detailed analyses on country-specific basis have only been recently begun (Thomson et al. 2007). The extent of genetic variability that exists in a gene pool is an important factor for genetic improvement in rice. Sri Lanka's rice gene pool consists of many abiotic and biotic stress tolerant traits with diverse agronomical characters (Ranawake and Amarasinghe 2014).

Oryza sativa varieties which have been grown in Sri Lanka from ancient times to the middle of the last century are known as traditional rice varieties. A landrace is a dynamic population of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted, and associated with traditional farming systems (Camacho Villa et al. 2005; Choudhury et al. 2013). Sri Lanka is considered as one of the secondary diversity centers for rice genetic resources (Kobayashi et al. 1991). In ancient times, farmers cultivated traditional rice varieties, due to their adaptability to Sri Lankan soil types, climate, geography, and harsh environmental conditions such as

flood, drought, soil salinity, iron toxicity, pests, and diseases. The traditional rice varieties have a historical origin, distinct identity, and lack formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems (Camacho Villa et al. 2005).

Rice varietal improvement by incorporating germplasm from traditional rice may lead to important advances as these varieties seem to harbor significantly higher genetic and phenotypic diversity than the cultivated rice (Bentota and Weerasinghe 2005; Atwell et al. 2014). Moreover, traditional rice varieties are one important component of the biodiversity of Sri Lanka.

Genetic fingerprinting of these varieties is essential to distinguish them and to characterize accessions. Molecular markers such as simple sequence repeats (SSR) and microsatellites are useful for assessing genetic variations within conserved gene pool in rice. To date, more than two thousand SSR markers of cultivated rice are available and these provide a powerful tool for studying close relatives. SSRs are simple, tandemly repeated, nucleotide sequence motifs flanked by unique sequences (Roa et al. 2000) and have become useful markers for genetic diversity analysis because they detect high levels of allelic diversity, occur frequently throughout plant genomes, and are easily assayed by PCR. SSR markers have been extensively used to identify genetic variation among rice species to analyze genetic structure within cultivated rice (Ren et al. 2005), and to evaluate genetic diversity among strains of wild rice (Shishido et al. 2006) and among cultivars of cultivated rice (Yu et al. 2003). Further, microsatellites have been used for studies of parentage (Roa et al. 2000), genetic mapping and breeding, gene flow, genetic diversity and population differentiation (Cho et al. 2000). Genetic differentiation among selected Sri Lankan traditional rice (*Oryza sativa*) varieties and wild rice species were conducted using AFLP markers by Rajkumar et al. (2011).

Despite the limited studies conducted, there is still a strong need for more detailed characterization of the responses and acclimatization mechanisms of rice under stresses that are occurring in farmers' fields. Thus, integration of crop agro-morphology and molecular approaches to dissect complex environment tolerance traits is essential. The objectives of the present study are; phenotypic characterization and Molecular analysis of traditional rice varieties collected from PGRC, Sri Lanka, and identification of a core collection of traditional rice varieties.

MATERIALS AND METHODS

Collection of seed material

One hundred (100) Sri Lankan traditional rice varieties collected from PGRC, Sri Lanka were used in the study.

Germination of seeds

Seeds were kept at 50 °C for 5 days to break the dormancy. Then seeds were kept in 70% alcohol for 2 minutes and washed properly with distilled water and dipped in 2% Clorox for about 30 minutes and again washed properly with distilled water. Then seeds were kept in an incubator at 35 °C for 7 days under dark conditions. The experiment was conducted at seedling stage. Germinated seeds were planted in plastic trays filled with soil collected from paddy fields.

Agro-morphological characterization

The study was carried out in a plant house at The Open University of Sri Lanka, Nawala in the Low country Wet Zone, Western Province of Sri Lanka. The average ambient temperature was 28-32 °C and average relative humidity was 80-85% in the experiment site during the experiment period. The soil was collected from paddy field and filled into pots (5 kg pot^{-1}).

The germinated seeds were allowed for 2 weeks for reaching the seedling stage and were planted in pots according to Randomized Complete Block design with 4 replicates and 5 plants per replicate. Fertilizer management and the other crop management practices were followed according to the recommendations of Department of Agriculture. Thirty-two (32) agro-morphological characters were observed/collected as explained in PGRC Characterization Catalogue on Rice Germplasm (1999).

Statistical analyses

Data collected on agro-morphological characters of traditional rice varieties were analyzed using different statistical procedures. Prior to the analysis, data were converted to unique type, *i.e.* Nominal data. Descriptive statistics included frequency analysis, and cross-tabulation. Inferential statistical analyses were carried out using χ^2 test and multivariate analytical techniques such as cluster analysis (CA) and Multidimensional scaling (MDS) to explore the statistical procedural variations in the outcomes. These procedures were used to examine the patterns of grouping of traditional rice varieties according to their agro-morphological features. Based on the grouping patterns reflected in the analyses, rice varieties were grouped into seven (07) categories such as A, B, C, D, E, F, and G (Table 1). In addition, classification and regression analysis was performed to extract the diagnostic agro-morphological features of the groups of traditional rice varieties include in the study.

Molecular characterization

A total of 100 traditional rice varieties collected from PGRC, Sri Lanka were used in the study. The green leaves were individually collected from 20-day-old seedlings for DNA extraction. The gDNA was extracted using the Plant genomic DNA kit (Biomed DL114-01) following by the CTAB protocol. Thirty-three microsatellite (Simple Sequence Repeat-SSR) primer pairs were used to assay genetic variation. The DNA amplification was carried out using a 2,720-thermal cycler (Applied Biosystems). PCR products were subjected to fragment analysis by capillary electrophoresis using an Applied Biosystems 3130×l DNA analyzer (Applied Biosystems).

RESULTS AND DISCUSSION

A long history of traditional rice production across diverse environments in Sri Lanka has led to a diverse array of traditional rice varieties. However, the relative importance and influence of yield-related traits on grain yield have changed over time due to rice improvement. The yield potential of a rice variety is a theoretical concept determined by a complex series of interactions with the components of the environment it is exposed to. Even though recommendations of crop varieties are done based on grain yield, there are other important traits related to grain quality and/or agronomy which are not related to grain yield (Samita et al. 2005). Under such circumstances, selection based on yield can lead to the loss of these important characters. Therefore, classification using multiple morphological characteristics is important to identify adaptation of a variety and to improve the evaluation of varieties for potential adaptation (Lin and Binns 1985; Lin et al. 1986). At present, there is still a lack of information on morphological diversity, how the diversity has changed with rice improvement, and its impact on grain yield of traditional and improved rice varieties in Sri Lanka.

Traditional rice varieties represent important genetic reservoirs with valuable traits and there is an urgent need to provide proper incentives and encourage the farmers to cultivate these to help in the *in situ* conservation of this important gene pool. The selected rice cultivars with abiotic stress tolerance have the potential for direct introduction as cultivars or utilization in the breeding programs. Sri Lankan traditional rice varieties were found to be biotic and abiotic stress tolerant showing exceptional levels of tolerance which play an important role in rice breeding programs (Madurangi et al. 2012; Munasinghe et al. 2017).

However, these varieties are rapidly being lost due to favor of agronomically improved rice varieties. Gene Bank of Plant Genetics Resource Center (PGRC) conserves a collection of over 2000 traditional rice accessions. Only a few studies have so far been conducted on genetic diversity among Sri Lankan traditional rice (Rajkumar et al. 2011; Ranawake and Amarasinghe 2014; Wijayawardhana et al. 2015; Bandara et al. 2017) and there is a need to conduct a comprehensive study.

Studies conducted by Wijayaeardhana et al. (2015) and Bandara et al. (2017) using Sri Lankan Traditional rice varieties revealed an effective use of agro-morphological characters based on Characterization Catalogue on Rice Germplasm descriptors to characterize Sri Lankan traditional rice varieties. A similar method was adopted in the present study using 32 Rice Germplasm descriptors explained in PGRC Characterization Catalogue on Rice Germplasm (1999) for agro-morphological characterization of 100 traditional rice varieties.

Microsatellites have been used for studies of genetic diversity and population differentiation in rice germplasm. Genetic differentiation among selected Sri Lankan traditional rice (*Oryza sativa*) varieties and wild rice species were conducted using AFLP markers by Rajkumar et al. (2011) and Molecular Characterization of Accessions from a Traditional Rice Cultivar, *Suwandel* using SSR markers by Gunasena et al. (2015). In the present study, 33 SSR markers were used to assay the genetic variation among 100 traditional rice varieties.

The present study revealed that traditional rice varieties represent distant clusters based on agro-morphological features, particularly on lemma-palea color, awn color at maturity, seedling height, and flag-leaf-angle. These findings were further confirmed by molecular studies indicating a comparatively high level of genetic differentiation among individuals of selected traditional rice varieties.

Agro-morphological characterization

Descriptive statistics indicated that variation of agro-morphological characters across rice varieties are negligible and certain characters such as presence/absence of awn, characteristics of awn are restricted to certain rice varieties.

However, result of the Kruskal-Wallis test showed that most of the agro-morphological characters significantly vary across rice varieties ($p < 0.05$). However, the variation of stem color among the rice varieties was not statistically significant ($p > 0.05$).

The result of CA and MDS indicated that majority of rice varieties are clustered into seven (7) groups in the dendrogram (Figure 1) and biplot of MDS dimensions and certain rice varieties indicated a grouping tendency. It is clear from the MDS biplot that the verities included in the study grouped into seven categories. The characters lemma-palea color, awn color at maturity, seedling height, and flag-leaf-angle are most likely determine the grouping in the dendrogram.

The characterization of these seven categories was confirmed by the results of CART (Classification and Regression Tree Analysis) analysis (Figure 2). Similar to clustering pattern of the dendrogram (Figure 1), according to the results of CART analysis, traditional rice varieties which consist of seven groups can be categorized by the characters; lemma-palea color, awn color at maturity, seedling height, and flag-leaf angle (Table 2).

Molecular characterization

According to the present study, all 33 loci displayed polymorphism (66.7-96.9 %) among 100 traditional rice varieties with a total of 387 alleles identified with an average of 11.72 alleles per variety. The AMOVA results showed that 34% of the variation distributed among accessions, 59% among individuals, and 7% within individuals indicating a comparatively high level of genetic differentiation among individuals of selected rice varieties. Structure analysis results illustrated that all 100 varieties were genetically structured into five (05) well-separated groups, high ΔK peak was recorded at K=15, K= 5, K= 19 and K= 2 respectively (Figure 3).

Table 1. Groups of traditional rice varieties resulted from the CA, PCA and MDS on agro-morphological data

Group and number of rice varieties	Rice variety (accession no.)
A (33)	*Murungabala wee* (3246), *Kanni murunga* (3260), *Periavellai* (3279), *Suduru samba* (3333), *Vanam* (3488), *Pokuru wee* (3499), *Maha ma wee* (3551), *Polon wee* (3553), *Muthu samba* (3564), *Pokkali* (3573), *El wee* (3578), *Seedevi* (3605), *Lumbini* (3613), *Maha wee* (3618), *Gal pa wee* (3341), *Kiri naran* (3350), *Kalundai* (3381), *Eth samba* (3383), *Moddai karuppan* (3388) *Wanni dahanala* (2053), *Hattapas dawas wee* (2051), *Dahanala* (2049), *Herath* (2048), *Bala samba* (2047), *Herath banda* (2063), *Handiran* (2057), *Gona baru* (2056), *Dingiri menika* (2055), *Demas* (2054), *Weda heenati* (2340), *Hal sudu wee* (2110), *Dik wee* (2109), *Heen dik wee* (3191)
B (03)	*Kalu heenati* (3471), *Pihatu wee* (3403), *Kurulu wee* (4903)
C (02)	*Kattagarang* (3176*), Rathu heenati* (3390)
D (02)	*Galu Sulai* (4616), *Thirissa* (3186)
E (02)	*Kalu hondarawalu* (4622*)* and *Yalalu* (4606)
F (27)	*Moothuki El* (3180), *Kuru hondarawalu* (3184), *Gangala* (3185), *Khombila* (3188), *Wanduru wee* (2046), *Balamurunga kayan (*2045), *Batapola wee* (2036), *Madayal (*3475), *Rankiri* (3476), *Sudugoda wee* (3477), *Japan sulai (*3393), *Devereddiri (*3398), *Atta wee* (02035), *Wanni heenati* (3401), *Batapola El* (2038), *Magoda El (*4905), *Nara wee* (4908), *Niyan wee* (4909), *Pathmawee* (4912), *Sudugalkada* (3983), *Welihandiran (*3916), *Mahamenik* (3923), *Manikkam* (3901), *Rath El* (3705), *Gambada Samba* (3714), *Goda heenati (*3724), *Kuruluthuda* (4553)
G (31)	*Soothuru wee* (3179), *HathiEl* (3183), *Molligoda* (4770), *kurulu wee* (4541), *MadaEl* (3177), *Yalihanthiran* (3187), *Pushmaraga* (3979), *Andikulan* (3189), *Mahakuru wee* (3190), *Puwakmalata samba* (3486), *Gires* (3193), *Katharamana* (3194), *Bala Murunga* (2108), *Hetada* (2069), *Heen murunga* (2979), *Mas samba* (2349), *Duru wee* (2990), *Polayal* (3071), *Thanthiri Balan* (3072), *Ratnawalu* (4916), *Weda heenati* (4917), *Pachchaperumal* (5383), *Black gora* (5387), *Malawariya* (5527), *Gallkatta* (3195), *Nandu heenati* (3197), *Arnolis wee* (3198), *Rathkuda* (3231), *Ralukuda* (3232), *Rathawalu* (3233), *Liyanweli* (4904)

Table 2. Characterization of groups of traditional rice varieties

Rice group	Flag-leaf-angle	Awn color at maturity	Lemma palea color	Seedling height (height class)
A	2	4	3	2
B	1	Absent	9	2
C	2	4	3	3
D	4	4	7	3
E	1	6	3	2
F	2	6	2	3
G	4	4	2	3

Note: Flag-leaf-angle 1. Erect, 2. Intermediate, 3. Horizontal, 4. Descending; Awn color at maturity 1. Straw, 2. Gold, 3. Brown (tawny), 4. Red, 5. Purple, 6. Black; Lemma Palea color 0. Straw, 1. Gold, 2. Brown spots on straw, 3. Brown furrows on straw, 4. Brown, 5. Reddish to light purple, 6. Purple spots on straw, 7. Purple furrows on straw, 8. Purple, 9. Black, 10. White; Seedling height (Height class) 1. < 20 cm, 2. >=20, 3. < 25 cm and 4. >=25 cm.

UPGMA analysis based on Jaccard's similarity separated the varieties into five (5) major clusters (Figure 4). A cophenetic correlation with r=0.786 strongly supported the clustering pattern of UPGMA dendrogram. A principal coordinate analysis (PCoA) also confirmed the UPGMA clusters. Varieties referred to the same cluster showed similar morphological characteristics (e.g. flag-leaf-angle, lema palea color, etc.) while varieties that are identified as morphologically distinct appeared genetically separated.

Breeding a new variety based on phenotypic characters may take more than a decade and, even then the release of an improved variety cannot be guaranteed. Molecular markers make this procedure more efficient and expedite the selection process in rice breeding. Hence, molecular markers are widely used as an efficient tool to characterize genetic variability. A similar study was conducted by Rajkumar et al. (2011) on the genetic diversity of 46 traditional rice (*Oryza sativa*) varieties in Sri Lanka using Amplified Fragment Length Polymorphism (AFLP) markers with ten primer combinations. A UPGMA analysis based on Jaccard's similarity separated the accessions into four major clusters. Traditional rice varieties referred to the same cluster showed similar morphological characteristics (e.g. height, grain color, etc.) while varieties that are known to be morphologically distinct appeared genetically separated.

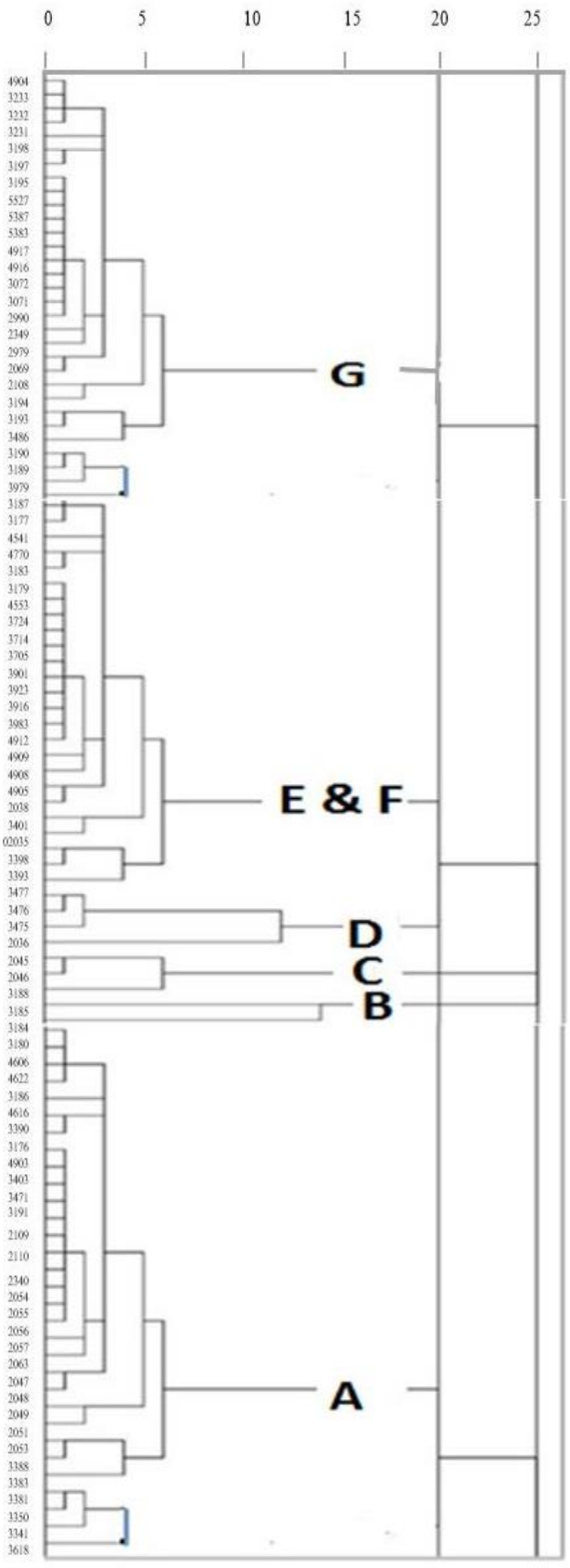

Figure 1. Dendrogram resulted from the agro-morphological characters of 100 traditional rice varieties

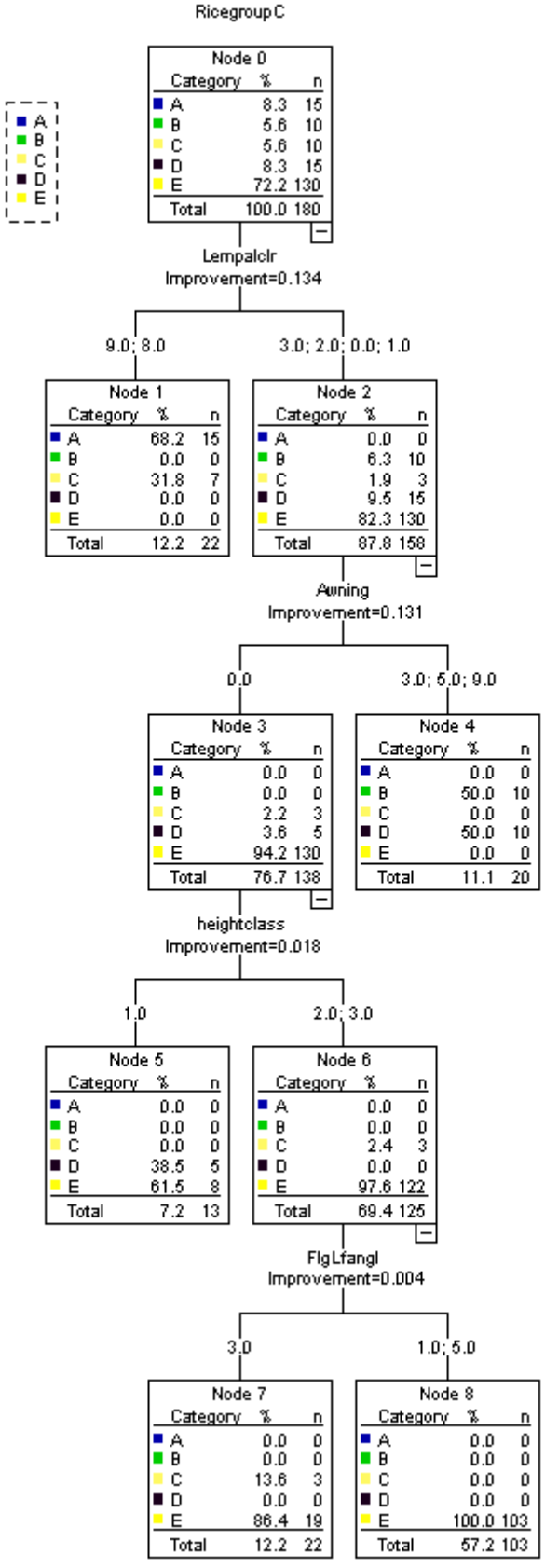

Figure 2. Summary of the characterization of the groups of rice varieties resulted from the CA and MDS Analysis using CART analysis

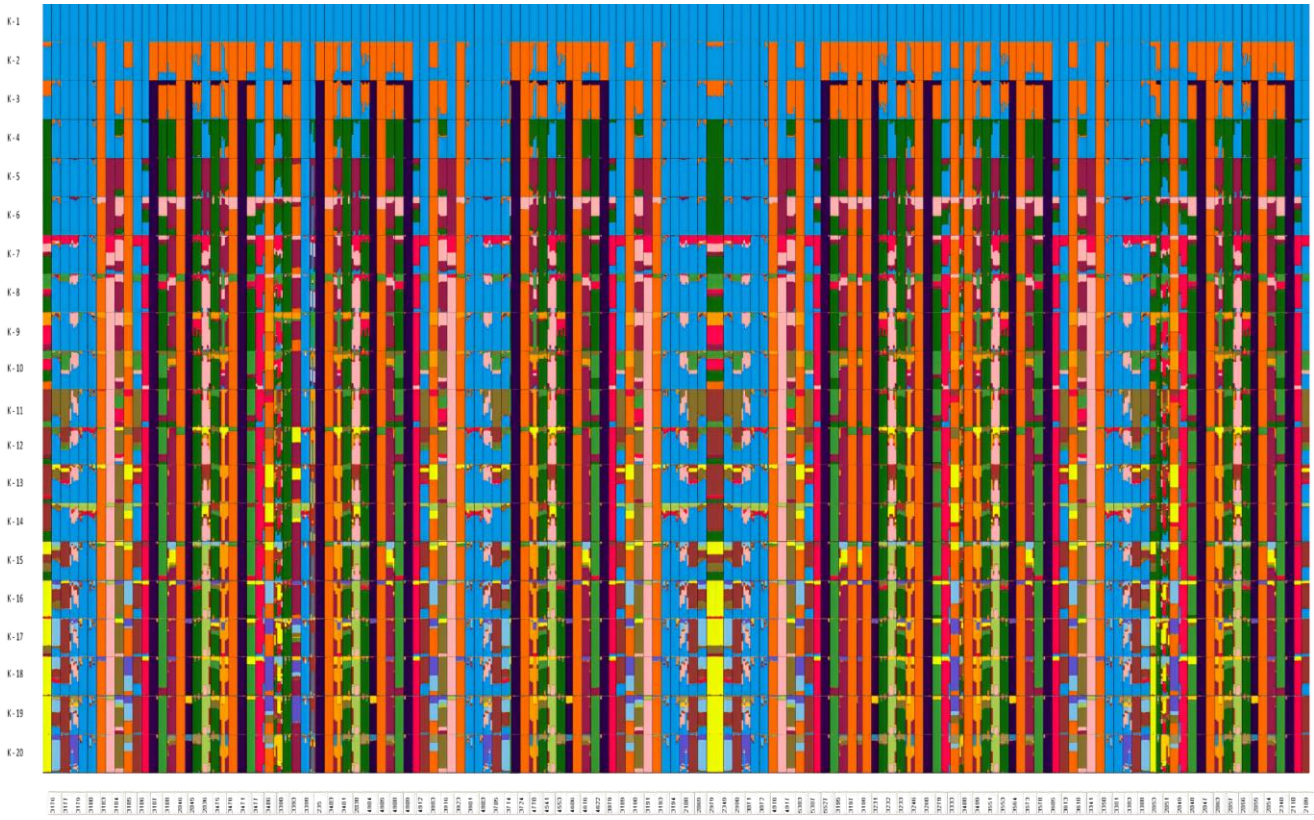

Figure 3. Structure results bimodal-based population assignment at *K* from 1 to 20. Each vertical bar represents an individual (100 varieties), with its assignment probability to genetic clusters represented by different colors

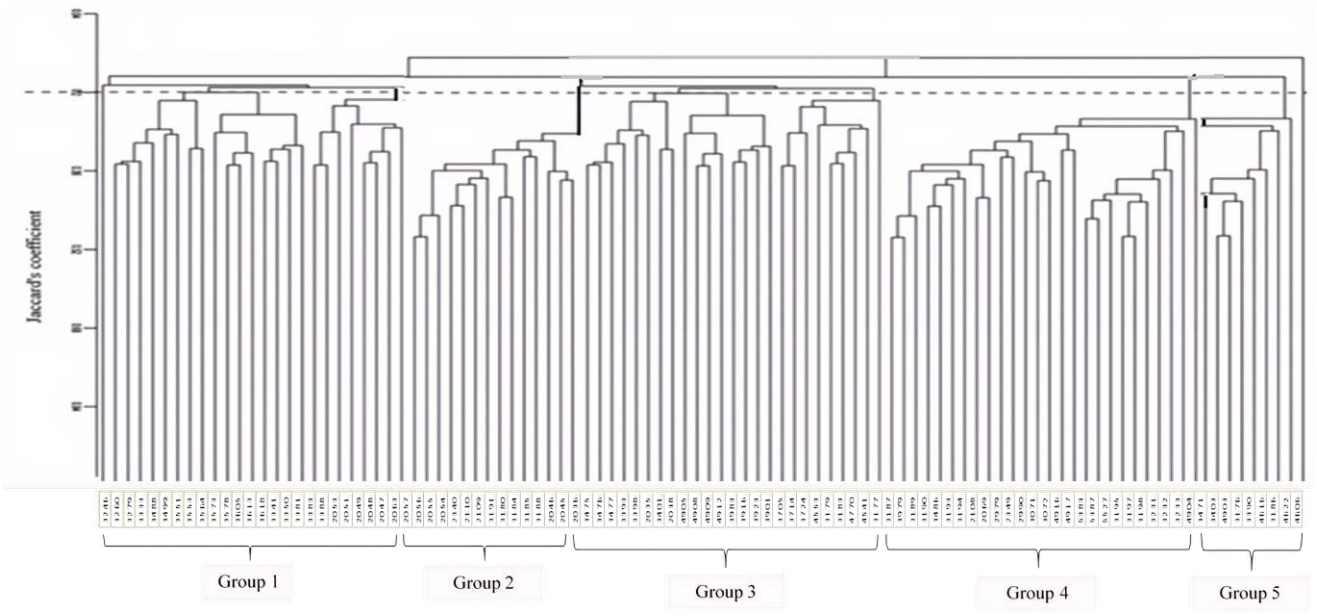

Figure 4. The UPGMA dendrogram showing genetic diversity among 100 Sri Lankan traditional rice varieties based on Jaccard's similarity coefficient. (Group 1-25 varieties, Group 2-14 varieties, Group 3-26 varieties, Group 4-26 varieties, Group 5-9 varieties)

In conclusion, the genetic diversity observed among the traditional rice varieties is significantly high. Genetic diversity assessment at agro-morphological level provides reliable information for the selection of germplasm to develop new rice varieties and in the conservation of traditional rice genetic resources for future breeding programs. The studied 100 traditional rice varieties consist of seven (07) groups which can be characterized by the

characters, lemma palea color, awn color at maturity, seedling height, and flag-leaf-angle. However, genetic diversity assessment at the molecular level provides reliable information for selection of germplasm in the development of new rice varieties and in conservation of traditional rice genetic resources. Structure analysis and UPGMA analysis based on Jaccard's similarity separated the tested 100 rice varieties into five (5) major clusters. All methods of analysis produced similar results confirming the reliability of data used in this study. Therefore, the genetic diversity information obtained will be useful in the efficient use of Sri Lankan rice germplasm collection in breeding programs. In addition, this information will be useful in management of *in situ* and *ex situ* germplasm collections in conservation programs for traditional rice varieties. Studies on genetic differentiation are also important to avoid duplication of traditional rice varieties in the Gene Bank at PGRC.

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