Research Paper

Morphological and Molecular Characterization of Traditional Rice Cultivar "*Hondarawala*"

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Abstract

Traditional rice cultivars are popular among farmers due to their grain quality characteristics and higher tolerance to drought, salinity, water logging conditions and low-fertile soils. Studies on available diversity within these cultivars provide useful information in varietal improvement in rice, when they are utilized as good sources of useful traits. In this study, genetic diversity of 21 accessions of *Hondarawala*, conserved at Plant Genetic Resources Centre (PGRC), Sri Lanka was assessed through morphological and molecular means. Thirty-nine morphological traits were assessed based on PGRC descriptors. A total of 30 SSR markers were used and genetic diversity was analyzed using Power Marker 3.25 version. Multivariable cluster analysis separated the selected germplasm into eight phenotypic groups at 82% similarity where 13 accessions clustered together, while other 8 accessions stayed separately. Molecular study revealed a total of 123 alleles across the tested accessions Polymorphic information content values ranging between 0.086 and 0.735 setting the mean at 0.505 and the allele number ranging from 4 to 6. Based on the genetic distances, these 21 accessions grouped in to 3 different clusters and 1, 14 and 6 *Hondarawala* accessions grouped in each cluster respectively. Ten accessions clustered together in morphological and molecular grouping and were identified as the representative sample of Hondaralawala. The results revealed that there is a considerable morphological and genetic diversity available among accessions of the same cultivar which makes it important in systematic conservation of the germplasm and provide information for utilization.

Keywords: Characterization, Genetic Diversity, *Hondarawala*, SSR markers, Traditional rice

Introduction

Rice is the third largest agricultural food commodity consumed over half of the world population. As the staple food of Sri Lanka, rice plays a significant role and the country has a self-sustaining rice production.

Before 1940s, only traditional rice cultivars (TRVs) were cultivated in Sri Lanka. Most of them were long aged, tall and low yielding but comparatively high in taste and rich in health functional properties. In early 1960, high yielding and fertilizer responsive newly improved rice varieties (NIVs) were introduced to the Sri Lankan paddy cultivation by focusing on large scale production to fulfill the demand arising from the increasing population (Rajapakse *et al*, 2000). Therefore, traditional rice cultivation was truncated, narrowed of genetic base and replaced by NIVs in order to attain higher yield and shorter life span. Although, NIVs fulfill the estimated production to a certain extent, traditional rice varieties have a significant demand due to their favorable agronomic, medicinal and nutritious qualities (Withanawasam, 2017).

At present, over 5000 rice accessions are available in the gene bank of Plant Genetic Resource Centre (PGRC), Gannoruwa. From the total collection 893 varieties belong to traditional rice and some are popular among farmers and consumers. *Suduru samba*, *Suwandal*, *Pachchaperumal*, *Hondarawala* and Kurulu thuda are some of the popular varieties. In 1960s, germplasm of Sri Lankan traditional cultivars were conserved in the IRRI gene bank and later replicas were placed back in the PGRC gene bank. However, traditional rice germplasm in PGRC have also been collected from different geo-graphical regions and cultivar names were derived from the information gathered from the farmers. Therefore, in the PGRC collection, there may be some mismatches such as duplicated accessions existing within a cultivar. Thus, morphological and molecular evaluation to identify duplicated or dissimilar accessions and selection of representative samples is essential in conserved seed storage of TRVs (Gunasena *et. al*., 2015; Withanawasam, 2017).

"*Hondarawala*" traditional rice cultivar is popular among traditional rice growers and has been used as a parental cultivar in rice breeding due to its high pest and disease tolerance. *Hondarawala* has been identified as a brown plant hopper resistant rice cultivar surviving under low fertile soils as well. It has been proven that *Hondarawala* possesses *PSTOL1* gene which confers tolerance to low soil phosphorus conditions (Kottearachchi and Wijesekara, 2013). Thus, this cultivar was used as a parent in the development of Bg 452, an improved rice variety released by RRDI, Batalagoda which is also moderately resistance for rice blast, bacterial leaf blight and gall midge department of Agriculture website. (www.. gov.lk).

The objective of the present study was to analyze morphological and molecular diversity of 21 *Hondarawala* accessions conserved in PGRC, to prove their available diversity with scientific evidence to be used in further utilization.

Materials and Methods

Morphological characterization

Twenty-one *Hondarawala* accessions conserved at the PGRC gene bank were used for the study (Table 1). The experiment was carried out at the plant house of PGRC, Gannoruwa, Sri Lanka. Ten seeds of each accession were germinated and transferred to 8 L pots filled with paddy soil. Trial was done using a completely randomized design with 3 replicates.

Twelve quantitative and 27 qualitative morphological characters were measured according to the PGRC descriptors developed based on the rice descriptors of International Plant Genetic Resources database (IPGRI database, 2017). Leaf characters (leaf length, leaf color, Leaf blade width, leaf angle, leaf pubescence, flag leaf angle), Ligule characters, Culm characters (culm length, culm diameter, culm angle etc.), Panicle characters (panicle length, panicle exertion, shattering etc.), awn characters, grain characters (grain color, grain weight, grain length, lemma and palea color, seed coat color, grain density, width and 100 grain weight), maturity age and panicle threshability were recorded.

Molecular characterization

Thirty-one SSR markers were used (Table 5) to analyze the genetic distance of 21 *Hondarawala* accessions with Bg 360 Newly Improved Variety (NIV) as the control. Genomic SSR primers were selected on the basis of high polymorphism information content. DNA was extracted from 14 days old rice seedlings using the optimized Cetyl Trimethyl Ammonium Bromide (CTAB) protocol at the molecular laboratory of PGRC. Polymerase Chain Reaction (PCR) was carried out for thirty SSR markers which were broadly dispersed through 12 chromosomes of rice genome (Siriwardhana *et al*., 2016; Manatunga *et al*., 2019; Koodalugodaarachchi *et al*., 2022).

Table 1. Selected *Hondarawala* accessions used for the study

PGRC accession No	IRRI reference number	PGRC accession No	IRRI reference number
003493	IRGC12075	004071	IRGC31416
003521	IRGC15181	004243	Not available
003528	IRGC15190	004731	IRGC50986
003678	IRGC15363	006197	Not available
003850	IRGC15567	006198	Not available
003867	IRGC15588	006199	Not available
003906	IRGC15634	006200	Not available
003911	Not available	006428	Not available
003977	IRGC15728	006690	IRGC12076
003988	IRGC15744	008499	Not available
004070	IRGC31415		

PCR mixture was prepared to 15 ul using 3 ul of 5X buffer, 1.20 ul of $MgCl_2$, 0.30 ul dNTP, 0.10ul taq polymerase, 3.00ul template DNA, 6.65 ul of distilled water and 0.375 ul of forward primer & reverse primers. PCR amplification was performed using Applied Biosystem thermal cycler. PCR programme was initial denaturation 94 °C, 1 min; denaturation 95 °C, 1 min; primer annealing 55 °C-65 °C, 1 min; final extension 72, 1 min. Amplified PCR products were confirmed using 1.0% agarose gel and the confirmed products were further separated using 8% non-denaturing polyacrylamide gel electrophoresis (PAGE). Unambiguous DNA bands were scored visually comparing 100 bp DNA size marker.

Data analysis

Morphological data analysis

Multivariate hierarchical clustering was carried out for thirty-two different quantitative morphological characters and standardized quantitative data. Seven characters were not account for the cluster analysis due to uniform characters across the tested accessions. Hierarchical clustering was done based on single linkage method using Euclidean distances.

Principal component analysis was done using Minitab version 15 (Minitab, 1991). Quantitative data and ranked qualitative data were used for cluster analysis.

Molecular data analysis

DNA band for each primer was evaluated using PowerMarker software (version 3.25) and the number of alleles for each marker, polymorphism information content (PIC), major allele frequency and the heterozygosity were calculated. The genetic distance matrix was generated from allele frequencies according to Nei *et al*., 1983. A phylogenetic tree was constructed based on the Nei's genetic distance matrix according to the neighbor joining method.

Results and Discussion

Morphological characterization

Most of the qualitative phenotypic characteristics studied such as sterile lemma color, ligule colour and shape, lemma and palea colour, leaf blade pubescence, seed coat colour and auricle color were more or less similar in almost all the accessions. Seed coat colour was white only in accession 006200 while all other 20 accessions were red. Awn was present only in five accessions namely 003906, 008499, 004243, 004731 and 003988 while others were awn-less (Figure 1).

Quantitative characters such as number of culms, culm length, days to heading, maturity age, leaf length and width, 100 grain weight grain length and width and panicle length showed significant diversity among accessions (Table 2). Maturity age differs 38 days between earliest and latest maturing accession.

Similarly, days to heading varied in between 57 and 108 days. Weight of 100 grains, which represents the size of the grain of each accession differs from 1.8 to 2.9 g (Table 2).

Table 2. Range of values of selected quantitative traits across tested *Hadarawala* accessions

Trait	Range of values of selected parameters
Leaf length	20 – 70 cm
Leaf width	9.0 – 15.0 mm
Number of culms	5-14
Days to heading	57 – 108 days
Ligule length	16.5 – 37.33 mm
Panicle length	18 - 29.83 cm
100 grain weight	1.8 – 2.9 g
Grain length	7.84 – 9.33 mm
Grain width	2.09 – 2.85 mm
Maturity age	113 – 151 days

In multivariate hierarchical clustering, distance value ranged from 48.79 - 91.19 %. Eight clusters were identified at the 82% similarity level and cluster 1, 2, 3, 5, 6 and Cluster 7 contained only one accession, 006428, 003977, 003906, 003911, 003867 and 008499 respectively. Two accessions included in cluster 4, namely 003988 and 004243. Majority of accessions numbering 13 were included in the Cluster 8, which can be considered as the main cluster. Individually clustered accession 006428 was the most distance accession to the main cluster. It showed the lowest mean values of leaf width, days to heading and maturity, panicle length and grain weight of 100 seeds to be clustered far apart. However, the accession with white seed coat was included in the main cluster (Figure 2). IRRI referenced accessions were scattered among clusters 2, 3, 4, 6 and 8 but not 1, 5 and 7 indicating the high level of diversity among collection of *Hondarawala* accessions conserved at IRRI as well.

Principal Component Analysis (PCA) revealed that seven principal components (PCs) had a cumulative variance of 76.80 % (Table 4). PC_1 accounted for 18.9 % variation from the total variability. PC_2, PC_3 and PC_4 accounted for variability of 14.10 %, 10.80% and 9.80%, respectively (Table 4). Altogether these first four PCs resulted for 53.60 % of total variance. Highly positive loading factors of the characters in the obtained PCs showed the morphological traits that contributed to total variation in the rice germplasm (Table 3). These traits were secondary branching, leaf blade pubescence, Ligule length, and culm angle and grain width with factor loadings of 0.346, 0.264, 0.194, 0.180 and 0.133, respectively (Table 4). These five phenotypic traits explained maximum variability (18.90 %) of PC_1. In PC_2, apical color, ligule length, grain width, culm diameter and leaf blade width showed higher loading factors for variation with 0.284, 0.278, 0.276, 0.236 and 0.229, respectively (Table 4), while it accounts for 14.10% of total variation.

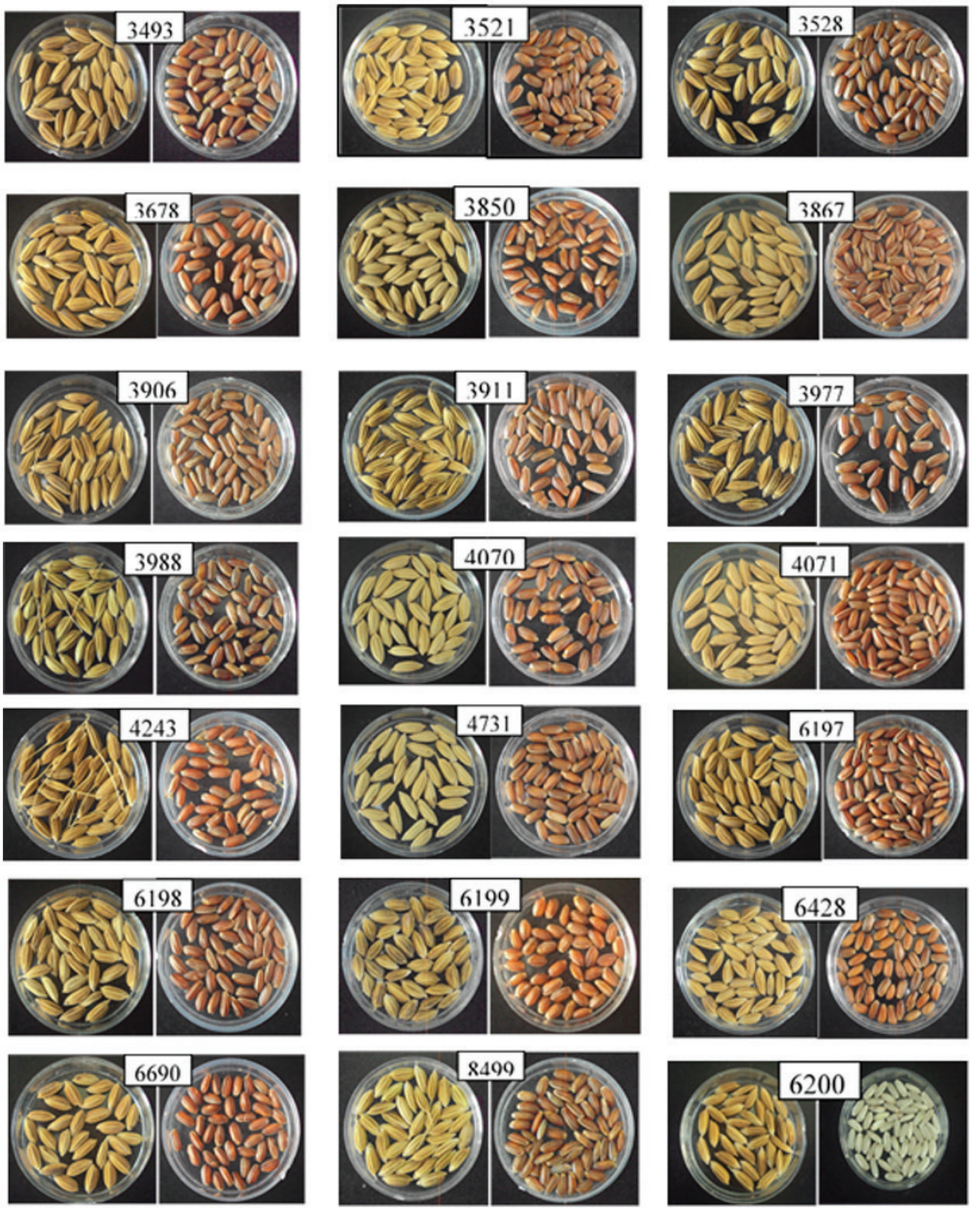

Figure 1. Grain diversity (lemma and palea colour, seed coat colour and presence of awn) of *Hondarawala* accessions studied.

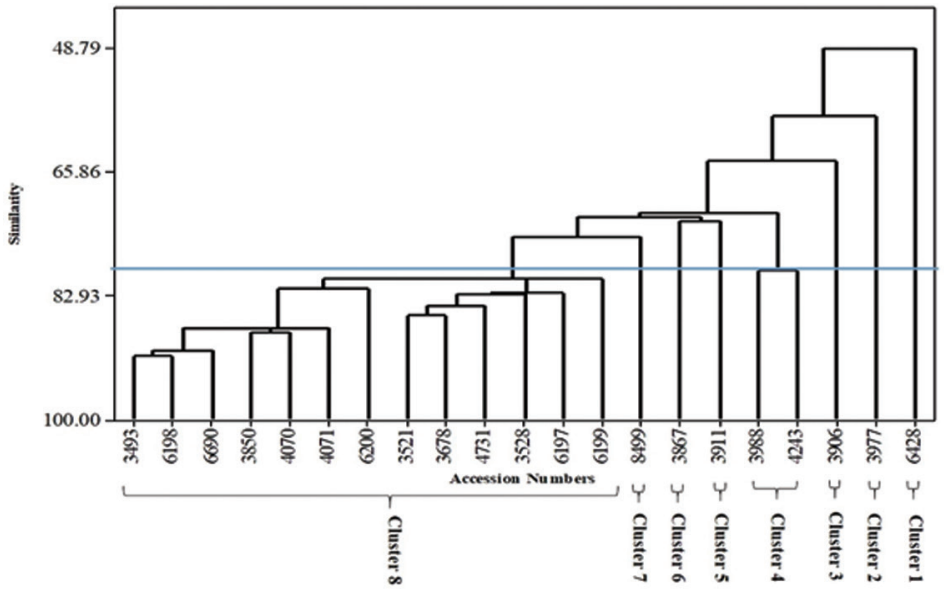

Figure 2. Dendrogram developed for different *Hondarawala* accessions based on morphological parameters using Euclidean distances and Single linkage method of Cluster Analysis

Table 3. Eigen values, explained variance and cumulative variance of Principal components

Component	Eigen value	Explained Variance (Percent)	Explained Variance (Cumulative)	Component	Eigen value	Explained Variance (Percent)	Explained Variance (Cumulative)
1	5.86	18.9	18.9	11	0.7	2.2	92.3
2	4.38	14.1	33	12	0.49	1.6	93.8
3	3.33	10.8	43.8	13	0.47	1.5	95.3
4	3.04	9.8	53.6	14	0.41	1.3	96.6
5	2.52	8.1	61.7	15	0.3	1	97.6
6	2.38	7.7	69.4	16	0.27	0.9	98.4
7	2.27	7.3	76.8	17	0.24	0.8	99.2
8	1.48	4.8	81.5	18	0.14	0.5	99.7
9	1.44	4.6	86.2	19	0.07	0.2	99.9
10	1.19	3.9	90	20	0.39	0.1	100

Table 4. Eigenvectors for the different morphological characters for principal components

Variable	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7
Leaf blade length	0.090	-0.277	-0.085	0.150	0.329	-0.153	0.202
Leaf blade width	-0.027	0.229	-0.007	0.122	-0.259	-0.243	0.259
Leaf blade pubescence	0.264	-0.171	0.070	-0.064	-0.118	0.243	0.194
Leaf blade color	0.051	0.124	-0.203	0.221	0.097	-0.199	-0.316
Leaf sheath color	0.035	0.191	-0.311	-0.017	0.217	0.038	0.157
Leaf angle	0.082	-0.264	-0.184	-0.092	0.013	0.025	0.119
Flag leaf angle	-0.011	0.119	-0.056	0.053	0.298	0.370	0.019
Ligule length	0.194	0.278	0.149	-0.139	-0.077	-0.209	0.042
Days to heading	-0.350	0.045	-0.012	-0.221	0.017	0.026	-0.086
Culm length	-0.340	0.149	0.023	-0.074	-0.067	-0.000	0.137
Culm number	-0.212	0.141	-0.069	0.238	0.091	0.266	-0.138
Culm angle	0.180	-0.110	-0.054	0.298	-0.056	0.297	0.053
Culm diameter	0.034	0.236	-0.295	-0.061	-0.068	-0.135	0.223
Internode color	0.034	-0.218	-0.157	0.143	-0.032	-0.283	-0.328
Culm strength	0.111	-0.010	-0.270	0.269	-0.278	0.181	0.050
Panicle length	-0.262	-0.087	0.070	0.156	0.198	-0.102	0.223
Secondary branching	0.346	0.031	0.181	0.001	-0.009	-0.216	0.053
Panicle exsertion	-0.013	-0.027	0.379	-0.137	-0.012	0.287	-0.205
Panicle axis at maturity	-0.269	-0.162	-0.030	-0.142	-0.222	-0.065	-0.176
Panicle shattering	-0.091	0.082	0.248	0.171	-0.223	0.220	0.273
Panicle threshability	0.157	-0.265	0.168	-0.226	0.009	0.071	0.007
Awning	-0.102	0.036	0.225	0.388	0.004	-0.102	-0.163
Awn color	0.056	-0.155	-0.271	-0.342	0.041	0.170	0.113
Apical color	0.074	0.284	-0.116	-0.064	0.255	-0.002	0.126
Lemma and palea color	0.050	-0.067	0.162	0.312	0.372	0.070	0.115
100 grain weight	-0.226	-0.081	0.145	-0.066	0.117	-0.154	0.372
Grain length	-0.135	-0.261	0.118	0.113	-0.087	-0.196	0.281
Grain width	0.133	0.276	0.042	-0.139	0.106	0.043	-0.015
Seed coat (bran) color	-0.007	0.009	0.207	-0.182	0.421	-0.134	-0.138
Leaf senescence	-0.097	-0.310	-0.258	-0.016	0.074	-0.044	-0.027
Maturity	-0.362	0.014	-0.130	-0.024	0.026	0.142	-0.006

Molecular characterization

Allelic diversity

Total of 123 alleles were observed and the mean allele number was 4.1 alleles per locus. RM217, RM224, RM228 and RM241 showed six alleles which was the highest allele number per locus (Table 5). The allele length for 30 SSR markers varied from 98-240 bp and the highest length 240 bp was recorded for RM560.

Table 5. Allelic diversity of 30 SSR markers on 21 *Hondarawala* rice accessions

Marker	No of alleles	PIC	MAF	Gene diversity	Marker	No of alleles	PIC	MAF	Gene diversity
RM20B	4	0.576	0.545	0.62	RM241	6	0.411	0.727	0.44
RM25	4	0.593	0.455	0.66	RM255	4	0.681	0.318	0.73
RM84	5	0.716	0.341	0.75	RM259	3	0.519	0.568	0.58
RM202	4	0.482	0.636	0.53	RM270	3	0.221	0.864	0.24
RM207	3	0.560	0.455	0.63	RM412	2	0.152	0.909	0.16
RM208	3	0.434	0.682	0.48	RM418	3	0.549	0.500	0.62
RM213	3	0.228	0.864	0.24	RM440	4	0.537	0.477	0.61
RM215	3	0.422	0.591	0.52	RM480	3	0.532	0.545	0.60
RM216	4	0.411	0.727	0.44	RM515	5	0.683	0.432	0.72
RM217	6	0.735	0.318	0.77	RM518	4	0.589	0.409	0.66
RM219	5	0.708	0.364	0.75	RM536	4	0.425	0.682	0.48
RM220	3	0.484	0.500	0.57	RM539	5	0.712	0.318	0.75
RM224	6	0.711	0.409	0.74	RM560	4	0.533	0.500	0.61
RM228	5	0.710	0.364	0.75	RM571	3	0.086	0.955	0.09
RM236	3	0.508	0.545	0.58	Mean	3.93	0.505	0.562	0.55
RM237	4	0.239	0.864	0.25					

PIC- polymorphic information content, MAF-Major allele frequency

The highest major allele frequency, 0.955 was produced by RM571 and all different markers were laid between frequency ranges of 0.318 to 0.955. PIC values ranged from 0.086 for RM 571 to 0.735 for RM 217 and the mean PIC was 0.505. The gene diversity varied between 0.088 for RM 571 and 0.770 for RM 217 (Table 5).

The majority of the markers i.e. 18 markers out of 30 showed PIC value higher than 0.5, explaining the high allelic diversity in markers used in this study. It suggests these markers can be used in future genetic diversity analysis of rice germplasm. *Hondarawala* showed diversity for different markers while previous studies have done for Sri Lankan traditional cultivars *Murungakayan* and Dahanala using the same set of SSR markers and had shown higher allelic diversity for different markers (Manatunga *et al*, 2019; Koodalugodaarachchi *et al*., 2022). Further, *Hondarawala* germplasm showed a significant gene diversity among the 21 accessions evaluated, ranging from 0.09 to 0.77 compared to cultivars *Murungakayan* (0.00–0.76) and Dahanala (0.00 – 0.74) (Manatunga *et al*., 2019; Koodalugodaarachchi *et al*., 2022). Allelic diversity derived in this study shows the suitability of selected markers to be used in future genetic diversity studies of traditional rice germplasm.

Population diversity

Pairwise genetic distance, estimated among twenty-one genotypes ranged from 0.1833 to 0.9000 (Table 6). Based on the dissimilarity matrix; Bg 360 and 006428 were the most genetically distant accessions showing 90% genetic distance as in morphological clustering since they were included in two varietal groups, NIV and traditional respectively. Among *Hondarawala* accessions 006428 was the genetically most distant accessions while 008499 and 006198 and 003493 were the most similar showing 86.6% and 18.3% dissimilarity respectively (Table 6).

Phylogenetic tree drawn based on molecular data grouped four major clusters at 0.1 distance (Figure 3). Bg 360 was clustered separately at the highest distance. This reveals that NIVs has not been conserved even mistakenly under the name of Hondarawla.

The accession 006428 was clustered separately in cluster 2 as the most distantly clustered accession from other accessions of *Handarawala*. Fourteen accessions (006199, 003528, 003493, 006198, 006690, 003850, 004070, 004071, 006200, 003521, 003678,003850, 003988 and 006197) were included in the cluster 3 and 10 accessions out of them were included in the morphological cluster 8 where 13 accessions were in that morphological cluster. Thereby, 003493, 006198, 006690, 003850, 004070, 004071, 006200, 003521, 003678 and 006197 belonged to morphological cluster 8 and molecular cluster 3. Interestingly, accession with white seed coat was clustered in this cluster 8 as in morphological clustering. It proves that the molecular characterization confirms the morphological characterization. Six accessions namely, 003867, 003977, 004731, 3911, 3906 and 4243 were clustered in Cluster 4 (Figure 3).

Diversity of Sri Lankan traditional rice cultivars namely *Suwandal, Pachchaperumal*, *Murungakayan*, *Pokkali, Kuruluthuda*, *Kaluheenati* and *Dahanala* have been assessed and they also had the significant genetic diversity within the same cultivar (Gunasena *et al*., 2015: Karunadasa and Samarsinghe, 2017; Manatunga *et al*., 2019; Koodalugodaarachchi *et al*., 2022).

There is no standard way of production of seed paddy for traditional cultivars in Sri Lanka. Cultivation of such cultivars by farmers has conserved the germplasm from generation to generation in the history. Since they produce their own seed paddy, standard procedures to avoid cross pollination and removing off types may not be properly practiced. This may lead to diversity among accessions coming under the same rice cultivar.

Accessions conserved in the PGRC is collected from different agro-ecological regions in the country. When *Hondarawala* accessions grown in such regions, they may develop some adaptive mechanisms suit to the area they are grown. This may lead to express some kind of diversity among accessions of same cultivar. The vast diversity shown in the accessions conserved under the same cultivar name can be due to cross pollination, natural mutagenesis, adaptation to grown environments, misnaming of cultivar or physical mixing of samples. Therefore, this type of study gives information to plant breeders, scientists, students or any other germplasm utilizers.

Table 6. Genetic distance matrix of tested accessions based on Nei's genetic distance

	3493	3521	3528	BG360	3678	3850	3867	3906	3911	3977	3988	4070	4071	4243	4731	6197	6198	6199	6200	6428	6690	8499
3493	0.0000																					
3521	0.4333	0.0000																				
3528	0.2833	0.4167	0.0000																			
BG360	0.7667	0.7667	0.7667	0.0000																		
3678	0.2500	0.4167	0.3167	0.8000	0.0000																	
3850	0.4167	0.4000	0.3667	0.8333	0.4833	0.0000																
3867	0.5833	0.6667	0.6000	0.7833	0.6000	0.4833	0.0000															
3906	0.5500	0.5667	0.6333	0.7000	0.5167	0.5500	0.6833	0.0000														
3911	0.6000	0.6000	0.6667	0.7333	0.5833	0.6333	0.4500	0.5167	0.0000													
3977	0.5667	0.7667	0.4833	0.7833	0.6500	0.6500	0.5833	0.5667	0.6000	0.0000												
3988	0.5667	0.3167	0.5333	0.7833	0.5167	0.4667	0.7167	0.6000	0.6667	0.7833	0.0000											
4070	0.4000	0.5167	0.3833	0.8000	0.5333	0.3500	0.5667	0.5667	0.6667	0.5167	0.6333	0.0000										
4071	0.5333	0.4333	0.5333	0.8500	0.5833	0.3667	0.5833	0.6333	0.7167	0.6667	0.5000	0.5667	0.0000									
4243	0.5833	0.5500	0.7000	0.7000	0.5000	0.6833	0.5500	0.3500	0.3500	0.6000	0.6500	0.6500	0.6167	0.0000								
4731	0.5833	0.7167	0.5500	0.8167	0.5333	0.6167	0.6000	0.5500	0.3833	0.4667	0.8000	0.5667	0.6500	0.3667	0.0000							
6197	0.2667	0.4000	0.2833	0.8167	0.2500	0.4500	0.5833	0.6333	0.6000	0.5333	0.5500	0.4000	0.5667	0.5667	0.5000	0.0000						
6198	0.1833	0.4833	0.3333	0.8333	0.3500	0.3333	0.6333	0.6000	0.7000	0.5833	0.5167	0.3500	0.5833	0.6833	0.5333	0.3167	0.0000					
6199	0.4667	0.5500	0.3667	0.7833	0.4833	0.4333	0.6000	0.6500	0.6333	0.5500	0.5667	0.5667	0.5167	0.6833	0.6333	0.4667	0.5000	0.0000				
6200	0.5000	0.5167	0.6167	0.7000	0.5333	0.5667	0.6000	0.5000	0.4833	0.6500	0.6000	0.5667	0.5333	0.5333	0.5500	0.5667	0.5833	0.6833	0.0000			
6428	0.7333	0.7833	0.7667	0.9000	0.6500	0.6833	0.7500	0.7500	0.7667	0.6167	0.7667	0.8333	0.7500	0.7500	0.7500	0.7333	0.8000	0.6333	0.7000	0.0000		
6690	0.5500	0.5000	0.5333	0.8000	0.5167	0.5000	0.6833	0.5833	0.6667	0.7000	0.2333	0.6333	0.4500	0.7000	0.7833	0.4667	0.5833	0.5667	0.5833	0.7333	0.0000	
8499	0.4667	0.4500	0.4833	0.7167	0.5000	0.5500	0.5833	0.6000	0.6500	0.7333	0.5000	0.6167	0.3833	0.6000	0.6667	0.4667	0.5500	0.5000	0.3833	0.8667	0.4333	0.0000

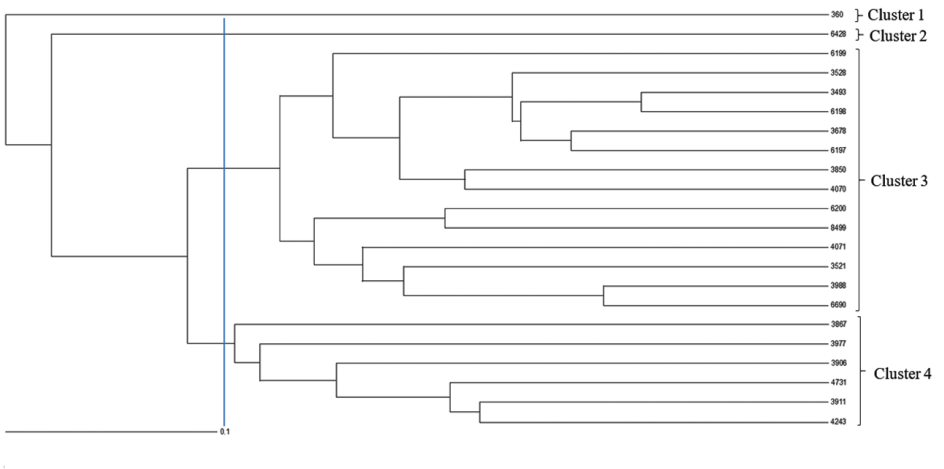

Figure 3. Phylogenetic tree derived based on Nei's genetic distance and Neighbour Joining algorithm

Conclusion

A significant degree of genetic diversity was found among accessions of *Hondarawala* traditional rice cultivar both morphological and molecular level. Ten accessions, 3493, 6198, 6690,3850,4070,4071,6200,3521,3678 and 6197 which were clustered together in morphological and molecular clustering, can be considered as the representative accessions of *Hondarawala* traditional rice cultivar. Data generated from this study could be used in rice breeding programs. Combination of molecular and morphological genetic diversity analysis provide precise set of information of diversity of germplasm.

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