# Genetic Diversity Analysis of Traditional Rice Variety 'Pachchaperumal' Using SSR Markers

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**Abstract**: Assessing the genetic diversity for miniaturization of crop collections to form mini core collection will lead to greater utilization of germplasm to develop improved crop cultivars along with effective maintenance of it. It has become vital to perform this for traditional Rice (Oryza sativa L.) varieties when considering the arising demand for their qualities. This study was conducted to assess the genetic diversity of twenty 'Pachchaperumal' traditional Rice accessions with two control varieties Bg360 and 'Suwandal' Rice, conserved at the Seed Genebank of Plant Genetic Resources Center, Gannoruwa, Sri Lanka. Morphological analysis was done using seven standard seed morphological descriptors of Rice and, Molecular analysis was done using 16 SSR primers. Statistical analysis was done for morphological and molecular data using MINITAB 15 and POPGENE 1.31 softwares respectively. Strategy of bulking accessions was tested in this study relying on seed morphological clustering and those bulks were used in the molecular analysis. Results revealed a significant degree of genetic diversity among tested accessions both morphologically and molecularly, and molecular results were more contributive. Two distinct accessions were identified as potential off types of 'Pachchaperumal', accession numbers 3752 and 5547. Cluster analysis based on morphological traits generated seven major clusters at 1.62 relative distance. Depending on the pattern of clustering, ten bulks were formed. The molecular analysis based on bulks, generated five clusters in a relative genetic distance of 9.0. Eleven accessions were distinguished as representative set out of all 20 accessions and according to passport data 3 of them were Bg accessions, 5550, 5549 and 5546.

**Index Terms**: Bulks, Genetic diversity, Germplasm, Oryza sativa, Pachchaperumal, SSR, Traditional Rice

## 1 Introduction

Rice is the most important cereal of the world providing 21% of global human per capita energy and 15% of per capita protein [1]. Rice (genus Oryza) has only two domesticated species Oryza sativa and Oryza glaberrima out of its twenty two species [2 and 3]. Oryza sativa is known as Asian rice and Oryza glaberrima is the African rice. Hence Oryza sativa is the major food crop for people in Asia and nearly 90% of the world's rice is produced and consumed in this region. Furthermore, rice is the staple food for nearly 2.4 billion people in Asia including China, Japan, some parts of India, Thailand, Sri Lanka, Bangladesh and many more [4]. Being the staple food in Sri Lanka, the Rice sown lands satisfy around 95 percent of the domestic requirement [5]. Rice varieties grown in Sri Lanka from ancient times to the middle of the last century are known as traditional rice varieties. Also known as cultivar and it is defined as an agricultural or horticultural variety or strain originated and persistent under cultivation.


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E.g. Suwandel, Pachchaperumal, Maa-Wee, Kuruluthuda, Rathdel and Madathawalu [6]. These traditional varieties are known for good taste, impressive cooking qualities, high nutritional values and high resistance towards diseases, pests and abiotic stresses such as adverse weather conditions, soil salinity, iron toxicity and floods. Meanwhile, old rice varieties have low yield and poor response to fertilizers [6 and 7]. Therefore, new varieties based on traditional Rice were started to develop mainly targeting high yield of rice. Even though, the valuable gene pool in traditional varieties has led them to be preserved in ex-situ genebanks for genetic characterization and further utilization in breeding. About 4000 accessions of rice are available in the seed genebank of Plant Genetic Resource Center, Gannoruwa [8]. There are large number of accessions available for one variety and large number of varieties available for one plant species as well. Sometimes two or more different accessions may be duplicates of the same, or one accession may be included in different types. Morphological characterization may not be adequate to characterize the accessions precisely. Therefore, molecular characterization or the genetic analysis is essential for correct identification, classification and nomenclature. Generally, morphological characterization of rice is carried out using characteristic features such as plant, grain and cooking quality traits. E.g. Days to flowering, panicle length and number of grains per panicle, grain length and breadth, grain weight, appearance of the husk and grain aroma [9]. Genetic diversity has been utilized and preserved partially during the process of domestication and cultivation and not even 15 percent of potential genetic diversity has been utilized in crop plants. The rice genome has twelve chromosomes. Molecular Marker Based Genetic Diversity Analysis (MMGDA) is capable of assessing changes in genetic diversity of rice over time and space [9, 10]. DNA markers are used for molecular characterization. E.g. Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphisms (SNPs) [11, 12]. The microsatellite markers

based on simple sequence repeats (SSRs) are preferred over other molecular markers due to their ease of application, high reproducibility, rapid analysis, low cost, easy scoring patterns and greater allelic diversity [13]. Also they are distributed relatively uniformly throughout the genome [11,13, 14]. This research project was carried out to analyze the genetic diversity of all conserved accessions (20 accessions) of traditional rice variety 'Pachchaperumal' using 16 optimized SSR markers. The morphological characterization of seeds followed by molecular characterization contribute to a precise identification of representative set of 'Pachchaperumal' which can be utilized in consequent studies and breeding programs. Numerous researches have been conducted on genetic diversity analysis of Sri Lankan traditional Rice varieties but a specified project for all available accessions of 'Pachchaperumal' Rice has not been addressed. Rathnathunga et al., (2016) [15] have studied some morphological and physiological characters of 13 accessions of 'Pachchaperumal' but not extended towards genetics. Hence, this particular study will be contributing towards the discovery of total genome of Pachchaperumal, and thereby to identify most representative accessions in order to develop a mini core collection of traditional Rice varieties in Sri Lanka.

## 2 METHODOLOGY

This study was conducted at Plant Genetic Center, Gannoruwa, Peradeniya, Sri Lanka. A molecular analysis and seed morphological characterization of 'Pachchaperumal' were carried out under the study to assess 20 available accessions of seeds of 'Pachchaperumal' in the Seed Genebank of Plant Genetic Resources Center (PGRC).

### 2.1 Morphological Analysis

Seven seed characters; grain shape, lemma and pales color, seed coat color, sterile lemma color, pubescence on lemma, grain width and grain length, which are given in International Plant Genetic Resources Institute (IPGRI) descriptors for Rice, were used to evaluate seed morphology of seeds. A comparison of seed morphology among 20 accessions used in the study is shown in the figure 1.The recorded results were analyzed using Minitab 15 version to obtain a dendogram. The resulted dendogram was used to group the 20 accessions into 10 bulks based on their clustering and distance, and those bulked accessions were preceded in the molecular analysis [16].


***Figure 1:*** *Comparison of Seed Morphology among Accessions*

### 2.2 Molecular Analysis

After the seeds were obtained from the PGRC Seed Genebank, they were placed about a day at room temperature as they were stored under freezing temperature conditions. They were cleaned well and about 50 seeds from each accession were soaked in distilled water in petri dishes for about one to two days. After the seeds have germinated, they were transferred to wet tissue papers in petri dishes labeled with accession numbers. Distilled water and Albert solution (1 g/450 mL) were added them daily for faster and healthier growth of seedlings. After two weeks of growth, the young juvenile leaves were harvested from each accession for DNA extraction.

#### 2.2.1 Extraction of Genomic DNA

DNA was extracted from harvested leaves using the modified CTAB method invented by Murray and Thompson (1980) [17] with the optimized protocol at PGRC. About 1-2

g of leaves were cut into small pieces and ground to a fine powder using liquid Nitrogen (-196°C) in a clean mortar and pestle. The product was then added to a sterilized vial (10 mL) containing 4.0 mL of pre-heated (65°C) 2% CTAB extraction buffer. Then 10.0 µL of 0.2% β-mercaptoethanol was added to the vial under the fumehood and incubated at 65°C for 30 minutes in a shaking water bath. Then the vials which were containing products were allowed to cool to room temperature. After that, an equal volume (4 mL) of Isoamyl alcohol/ Chloroform mixture (1:24) was added under the fumehood. Next the vials were slowly shaken well in a shaker for about 10 minutes. Those were then centrifuged at 8000 rpm for 15 minutes. At this stage the mixture was separated into two clear layers and the supernatant was carefully transferred into a new labeled vial without disturbing the interface and lower layer was discarded. 1/10th volume (0.4 mL) of 10% CTAB was added to vials and then again added with 4 mL Isoamyl alcohol: Chloroform (1:24) mixture. They were again slowly shaken for about 10 minutes and spun at 8000 rpm for 15 minutes. The supernatant was again transferred into another new labeled vial. About 2/3rd volume (3 mL) of ice-cold Isopropanol was added to the vial gently mixing the contents by inverting. Thread-like white DNA pellets were observed at this stage and they were spooled out carefully into sterilized eppendorf tubes using pipette. If fine pellets were not obtained, those vials were kept at -20°C for few hours to overnight, spun at 10000 rpm for 10 minutes at 4°C and DNA pellets were gained. DNA pellets that spooled out or precipitated at centrifugation, were then added with 100 µL of 70% DNA washing alcohol and centrifuged at 10000 rpm for 5 minutes to wash the pellets and remove residual CTAB and other impurities. Then, ethanol was carefully drained out inverting the tubes and let air-dry. After alcohol was fully evaporated, finally DNA suspension buffer TE was added to tubes according to the size of pellets (Generally about 100-150 µL of TE was added to larger pellets). After adding TE buffer the tubes were placed at room temperature for overnight to re-suspend the DNA pellets. Pure fine DNA extractions were dissolved well and the suspensions were observed transparent without any residues suspended or precipitated. DNA suspensions were stored at -20°C for further usage.

### 2.2.2 DNA Confirmation, Quantification and Dilution

DNA confirmation, quantification and dilution were done by running the DNA in 0.8% Agarose Gel Electrophoresis (60 V, 1 hour) [18]. Thereafter, the gels were stained in 0.5 µL/mL Ethidium bromide and observed under UV light in Bio Rad Gel Documentation System. Afterwards, DNA was roughly quantified comparing band intensities with standard DNA ladders. Based on intensity of bands, the DNA was diluted using TE buffer to about 15 ng/µL. This final dilution value was obtained by a series of dilutions proceeded such as 5×, 10×, 40×. Generally, 3 µL volume was loaded from diluted DNA.

### 2.2.3 Polymerase Chain Reaction (PCR)

PCR was done using protocol optimized for Rice at PGRC. 10 µL PCR reaction mix was prepared (Table 1) using 15 ng/µL concentrated DNA of 10 bulked rice samples, PCR buffer, $MgCl_2$, dNTP, 16 SSR forward and reverse primers, sterile distilled water and taq DNA polymerase enzyme.

***Table 1:*** *Composition of 10 µL of PCR cocktail mixture*

| Chemical ingredient | Volume added for 10 µL PCR reaction (µL) |
|---|---|
| 5× PCR buffer | 2.0 |
| 25 mM $MgCl_2$ | 1.0 |
| 10 mM dNTP | 0.2 |
| 20 µM/µL forward primer | 0.3 |
| 20 µM/µL reversed primer | 0.3 |
| 5 unit taq polymerase | 0.05 |
| Sterile distilled water | 4.15 |
| DNA template | 2.0 |

Also, Bg 360 control and traditional rice 'Suwandal' Ac#10729 were amplified with 16 SSR primers to use as controls in the analysis. RM259, RM213, RM571, RM241, RM440, RM480, RM217, RM412, RM418, RM560, RM201, RM216, RM536, RM202, RM224 and RM270 were used as SSR markers in the study [19]. PCR amplification, which was invented by Kary B. Mullis in 1983 [20], was performed using thermocycler model 'Applied Biosystems (model# 9902)'. Each PCR amplification consisted of an initial denaturation step at 94°C for 4 minutes followed by a process of 35 cycles consisted of 3 steps namely, a denaturation step at 95°C for 1 minute, annealing step at 55°C for 1 minute and an extension step at 72°C for 2 minutes. At the end of the final cycle, final extension was carried out at a temperature of 72°C for 7 minutes, with subsequent holding temperature at 4°C. The whole PCR amplification session was done at annealing temperature 55°C using optimized PCR program for Rice at PGRC [21].

### 2.2.4 Analysis of PCR Products using Polyacrylamide Gel Electrophoresis (PAGE)

After the confirmation of amplified PCR products by 1.5% Agarose gel electrophoresis [21], they were analyzed by 8% non-denaturing Polyacrylamide Gel Electrophoresis in 1X TBE buffer (Pre-run at 200 V, 36-60 mA for about 1 hour and then PCR products run for 2-3 hours with DNA ladders 100 kb and 25 kb. Gels were stained in 0.5 µg/mL Ethidium bromide [22, 23], and then DNA bands were visualized using BIORAD Gel documentation system.

### 2.2.5 Expansion of DNA Bulks

After assessing the bulk PAGE images, the bulk DNA bands which were having multiple bands were expanded [24]. So the component individuals were separately amplified with corresponding primers and run on PAGE and the bands were scored separately in order to replace their bulk bands. In this, bulk 04 sample was expanded into it's components 11 and 18 under primers RM216, RM571, RM440, RM270 and RM202 and bulk 08 sample was expanded into it's components 03 and 07 under primers RM270 and RM202.

### 2.3 Data Scoring and Analysis

The DNA bands in resulted PAGE gel images were scored manually. Then the data was analyzed using Popgen version 1.31 to estimate the genetic diversity among tested bulked samples. The representative set of 'Pachchaperumal' Rice and any duplicates were identified considering both molecular and morphological clusters.

## 3 Results and Discussion

### 3.1 Seed Morphological Analysis

The results showed a considerable level of variability among accessions. Most accessions had brown furrows on yellowish background and few had brown furrows on gold background. Short hairs on lemma and palea were there in the majority of seeds. Seed width varied from 2.928 to 3.45 (mm) and length from 7.36 to 8.672 (mm). In addition, seeds were spindle shaped, had red seed coat color and straw color sterile lemma. Based on seed morphological results, two distinctly different accessions, Ac# 03752 and Ac# 05547 were identified as off types of 'Pachchaperumal' and were excluded from the molecular study and bulking. They had distinctly different straw colored lemma and palea, and higher values for seed length or width. Ac# 03752 possessed comparatively longer seeds of 8.672 mm while Ac#05547 owned comparatively thinner seeds of 2.928 mm.

#### 3.1.1 Cluster Analysis based on Seed Morphological Characters

Cluster analysis of seed morphology was completed using Minitab version 15 and has shown below (figure 2) is the dendogram obtained. Accessions 1 to 20 in the figure are 3429, 8827, 5383, 3408, 3752, 10734, 9049, 5547, 5548, 3946, 6834, 5549, 10749, 3937, 5546, 3879, 3136, 10767, 4641 and 5550 respectively. In the dendrogram, all the 20 accessions were clustered into 7 groups stating a relative distance of 1.62. Significantly, 12 accessions (According to the figure 2 respectively from left to right, accessions 3408, 3429, 5550, 10734, 3937, 3946, 4641, 5546, 5549, 6834, 10767 and 5548) were stick to one group illustrating potential closer relationship among them. 15 and 12 sample numbers (respectively accessions 5546 and 5549) have the closest morphological similarities. Four accessions have clustered independently and distantly (accessions 3136, 8827, 5547 and 10749) and the most distantly clustered individual was identified as accession 3136 with a relative distance of 4.86. The distantly related accession 5547 (sample number 08) was also identified as an off type which is separating from other narrow-down clusters at a relative distance of 3.24. The other accession 3752 (sample number 05) which was identified as an off type is separating from other narrow-down clusters at a slightly less distance than 3.24. Based on the relative distances among 20 individual accessions, all of them were bulked into 10 groups.

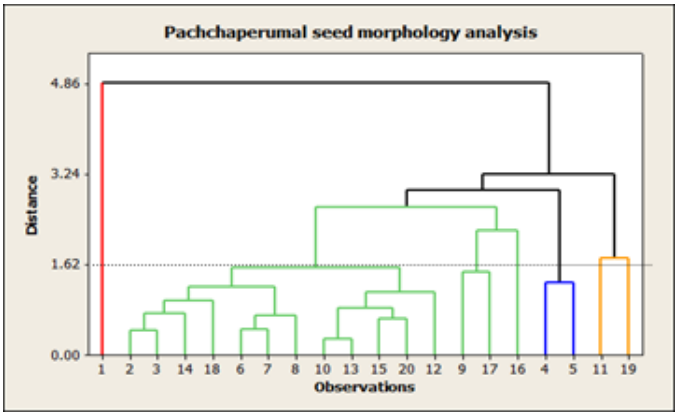
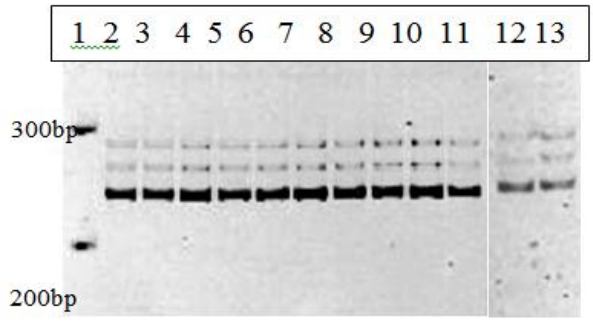



***Figure 2:*** *Dendogram of Rice Accessions based on Seed Morphological Characters and bulking*

### 3.2 Molecular Analysis

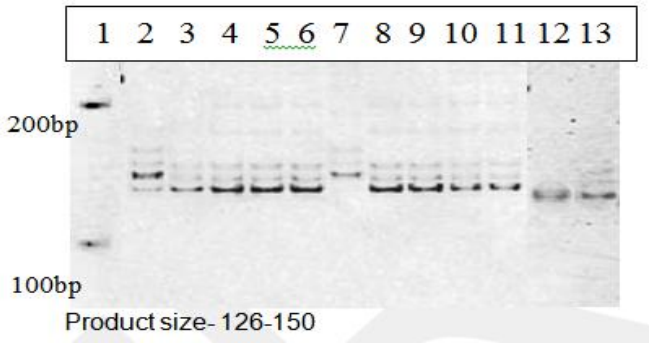
A considerable degree of genetic diversity was found out in the molecular analysis among the bulks tested based on SSR polymorphism. The extracted raw DNA was diluted (upto 15 ng/µL) with high dilution factors after they were quantified, in order to decrease the RNA bulks associated with raw DNA. This is essential because high RNA contents in DNA samples are interrupting the downstream processes PCR, PAGE and their results [21]. Few PCR amplicons which were resolved in 8% Polyacrylamide gels with respect to different SSR loci are given in figure 3.

***(a)*** **RM560**




Product size- 224-249
Alleles- 3
Lanes: 1-100kb DNA ladder, 2-B10 (Bulk sample), 3-B9, 4-B8, 5-B7, 6-B6, 7-B5, 8-B4, 9-B3, 10-B2, 11-B1, 12-Bg360, 13-Suwandal

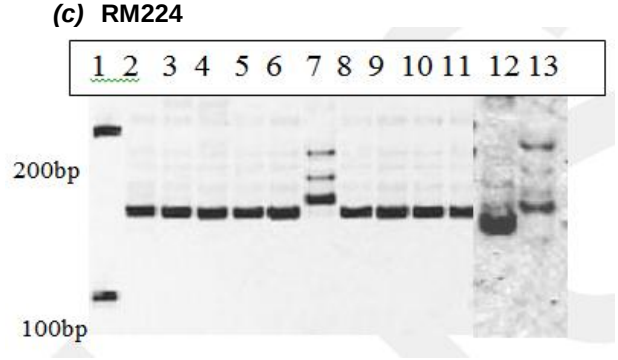
***(b)*** **RM213**




Alleles- 4

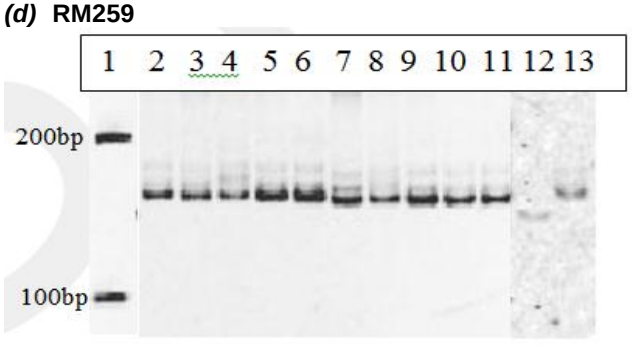
Lanes: 1-100kb DNA ladder, 2-B10, 3-B9, 4-B8, 5-B7, 6-B6, 7-B5, 8-B4, 9-B3, 10-B2, 11-B1, 12-Suwandal, 13- Bg360

***(c)* RM224**




Product size- 24-142
Alleles- 3
Lanes: 1-100kb DNA ladder, 2-B10, 3-B9, 4-B8, 5-B7, 6-B6, 7-B5, 8-B4, 9-B3, 10-B2, 11-B1, 12- Suwandal, 13- Bg360

***(d)* RM259**




Product size- 148-176
Alleles- 3
Lanes: 1-100kb DNA ladder, 2-B10, 3-B9, 4-B8, 5-B7, 6-B6, 7-B5, 8-B4, 9-B3, 10-B2, 11-B1, 12- Suwandal, 13- Bg360

***Figure 3:*** *Ethidium Bromide Stained Polyacrylamide Gel Images of PCR Products of the Primers; (a) RM560, (b) RM213, (c)RM224 and (d)RM259.*

However, stutter bands were observed even though attempts were taken to diminish them. Stutter bands are artefacts produced by DNA polymerase slippage [25]. Working on the purpose to reduce stuttering, first the PCR reaction volume per single reaction was reduced to 10 µL and next the 5 unit Taq DNA polymerase volume was restricted to 0.05 µL. This low amount of Taq was causing adequate polymerization of all samples, even though was not able to deplete stutters completely. 20 µM/µL forward and reverse primers were used in 0.3 µL volumes mostly, rather than 0.2 µL since sometimes higher volume was needed for amplification of whole sets.The number of alleles detected for all loci ranged from 2 to 4 deducing low levels of polymorphism of alleles.

**3.2.2 Cluster analysis based on SSR marker analysis**

According to the cluster analysis of polymorphic co-dominant data which were processed in Popgen V.1.31, a dendogram was resulted shown in figure 4 and the Nei's (1972) relative genetic distances are stated in table 2.

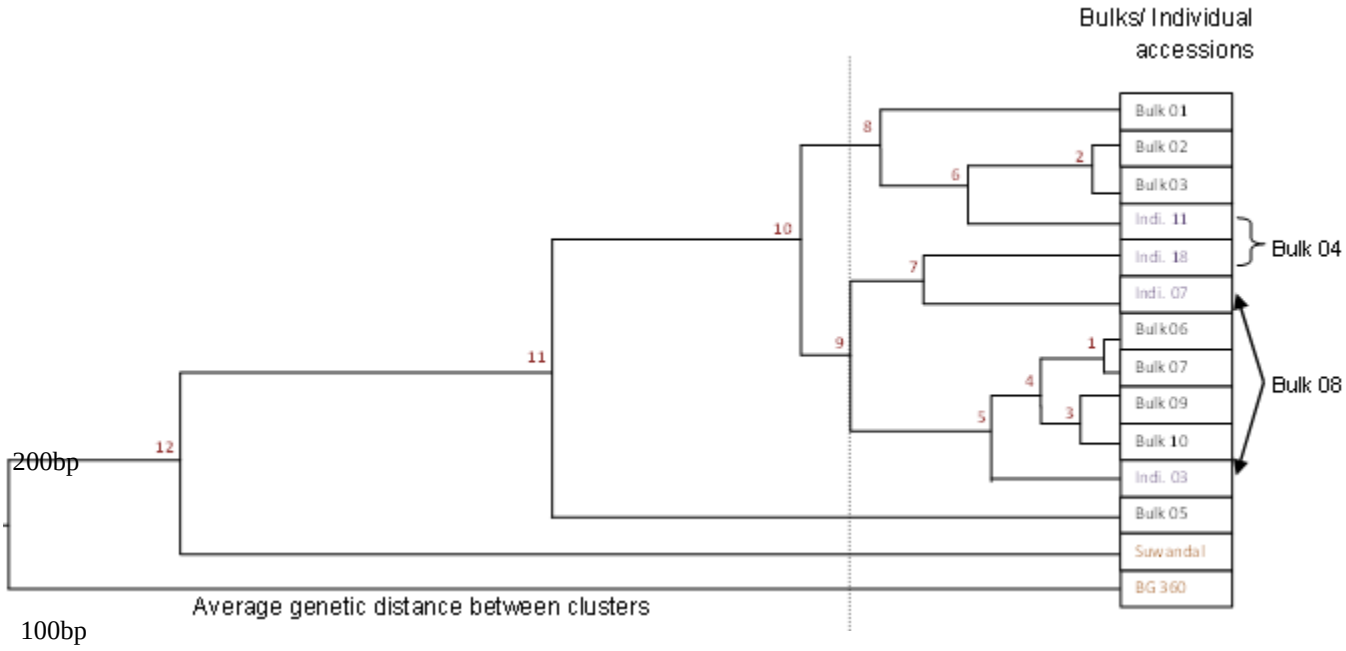



***Figure 4:*** *Genetic Diversity among 10 Bulk Samples based on Analysis of SSR Data Set; Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA, Modified from NEIGHBOR procedure of PHYLIP Version 3.5*

***Table 2:*** *(a) Dendrogram based on Nei's (1972) Relative Genetic Distance. (b) The samples represented by population ID*

***(a)***

| Between | And | Length |
|---|---|---|
| 13 | 12 | 11.35057 |
| 12 | 11 | 30.84015 |
| 11 | 10 | 16.51440 |
| 10 | 8 | 3.69451 |
| 8 | pop1 | 14.27582 |
| 8 | 6 | 7.33398 |
| 6 | 2 | 3.60727 |
| 2 | pop2 | 3.33457 |
| 2 | pop3 | 3.33457 |
| 6 | pop4 | 6.94183 |
| 10 | 9 | 3.22512 |
| 9 | 7 | 3.98964 |
| 7 | pop5 | 10.75557 |
| 7 | pop10 | 10.75557 |
| 9 | 5 | 8.45945 |
| 5 | 4 | 0.62176 |
| 4 | 1 | 5.66400 |
| 1 | pop7 | 0.00000 |
| 1 | pop8 | 0.00000 |
| 4 | 3 | 1.24352 |
| 3 | pop11 | 4.42049 |
| 3 | pop12 | 4.42049 |
| 5 | pop9 | 6.28576 |
| 11 | pop6 | 34.48473 |
| 12 | pop14 | 65.32488 |
| 13 | pop13 | 76.67545 |

***(b)***

| Population ID | Sample |
|---|---|
| 1 | Bulk 01 |
| 2 | Bulk 02 |
| 3 | Bulk 03 |
| 4 | 11 |
| 5 | 18 |
| 6 | Bulk 05 |
| 7 | Bulk 06 |
| 8 | Bulk 07 |
| 9 | 03 |
| 10 | 07 |
| 11 | Bulk 09 |
| 12 | Bulk 10 |
| 13 | BG360 |
| 14 | Suwandal |

All the tested bulks and expanded individuals were basically clustered into 5 groups at a relative genetic distance of 9 isolating individual clusters bulk 05, 'Suwandal' and Bg360. Bg360 and 'Suwandal' have been clustered separately apart from tested accessions. Out of these two, Bg360 has clustered more distantly at a relative genetic distance of 13 (76.67545 according to table 2(a)). Bulk 05 (Ac#5383) is the most distantly related sample to others indicating a relative genetic distance of 34.48473. Bulk 06 and 07 are having the closest genetic relationship and interestingly it is stated as 0.0 according to the Nei's values of relative genetic distance, inferring bulk 06 (Ac#3136) and 07 (Ac#3879) are duplicates based on 16 SSR loci tested. Interestingly, 11 and 18 individuals of bulk 04, and 03 and 07 individuals of bulk 08 have been clustered in different groups separately. In essence, these results are different from morphological results. The resulted pairwise comparisons of genetic identity and genetic distance are shown in table 3. The table is further elaborating the results of dendrogram stating the most identical pair (genetic identity measure 1.0000 in between) as population 7 and 8 (bulk 06 and bulk 07) and genetic distance as 0.0000. Least identical pair (genetic identity measure 0.3335 in between) or most genetically distant pair (genetic distance measure 1.0981 in between) has been indicated as populations 06 (bulk 05) and 01(bulk 01). Pairwise measurements between each tested accession and population 13 (Bg360) and between population 14 (Suwandal) are comparatively high values according to the table 3. Then all the bulks were expanded literally and analyzed to result a dendrogram which is shown in figure 5 and analogous with the dendrogram obtained for bulks. It was generally clustered into 7 groups at a relative genetic distance of 13 with same 3 individual clusters as in figure 4. The most closely related individuals are accessions 3429 and 3408 which are components of bulk 01. The most distantly separated accession is 5383, same as in figure 4.

***Table 3:*** *Nei's Original Measures of Genetic Identity and Genetic Distance Table; Nei's genetic identity (above diagonal) and genetic distance (below diagonal).*

| Pop ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | **** | 0.8065 | 0.7419 | 0.7097 | 0.6129 | 0.3335 | 0.6774 | 0.6774 | 0.6774 | 0.6129 | 0.5902 | 0.6003 | 0.2903 | 0.2335 |
| 2 | 0.2151 | **** | 0.9355 | 0.9032 | 0.7419 | 0.4002 | 0.8065 | 0.8065 | 0.6774 | 0.6774 | 0.7214 | 0.7004 | 0.3226 | 0.1668 |
| 3 | 0.2985 | 0.0667 | **** | 0.8387 | 0.6774 | 0.4669 | 0.7419 | 0.7419 | 0.6129 | 0.6129 | 0.6558 | 0.6337 | 0.2581 | 0.1668 |
| 4 | 0.3429 | 0.1018 | 0.1759 | **** | 0.8065 | 0.5003 | 0.8387 | 0.8387 | 0.7097 | 0.7097 | 0.7542 | 0.7337 | 0.2903 | 0.2335 |
| 5 | 0.4895 | 0.2985 | 0.3895 | 0.2151 | **** | 0.6670 | 0.6774 | 0.6774 | 0.6774 | 0.8065 | 0.7870 | 0.7671 | 0.1613 | 0.3002 |
| 6 | 1.0981 | 0.9157 | 0.7616 | 0.6926 | 0.4049 | **** | 0.5003 | 0.5003 | 0.5003 | 0.5003 | 0.6272 | 0.6207 | 0.1668 | 0.3103 |
| 7 | 0.3895 | 0.2151 | 0.2985 | 0.1759 | 0.3895 | 0.6926 | **** | 1.0000 | 0.8710 | 0.7419 | 0.8854 | 0.9005 | 0.2581 | 0.2668 |
| 8 | 0.3895 | 0.2151 | 0.2985 | 0.1759 | 0.3895 | 0.6926 | 0.0000 | **** | 0.8710 | 0.7419 | 0.8854 | 0.9005 | 0.2581 | 0.2668 |
| 9 | 0.3895 | 0.3895 | 0.4895 | 0.3429 | 0.3895 | 0.6926 | 0.1382 | 0.1382 | **** | 0.8065 | 0.8854 | 0.9005 | 0.1290 | 0.4002 |
| 10 | 0.4895 | 0.3895 | 0.4895 | 0.3429 | 0.2151 | 0.6926 | 0.2985 | 0.2985 | 0.2151 | **** | 0.7542 | 0.8338 | 0.1290 | 0.3335 |
| 11 | 0.5272 | 0.3265 | 0.4219 | 0.2821 | 0.2395 | 0.4665 | 0.1218 | 0.1218 | 0.1218 | 0.2821 | **** | 0.9154 | 0.1967 | 0.3051 |
| 12 | 0.5103 | 0.3561 | 0.4562 | 0.3096 | 0.2651 | 0.4769 | 0.1048 | 0.1048 | 0.1048 | 0.1818 | 0.0884 | **** | 0.2001 | 0.3793 |
| 13 | 1.2368 | 1.1314 | 1.3545 | 1.2368 | 1.8245 | 1.7912 | 1.3545 | 1.3545 | 2.0477 | 2.0477 | 1.6258 | 1.6089 | **** | 0.2668 |
| 14 | 1.4547 | 1.7912 | 1.7912 | 1.4547 | 1.2034 | 1.1701 | 1.3212 | 1.3212 | 0.9157 | 1.0981 | 1.1870 | 0.9694 | 1.3212 | **** |

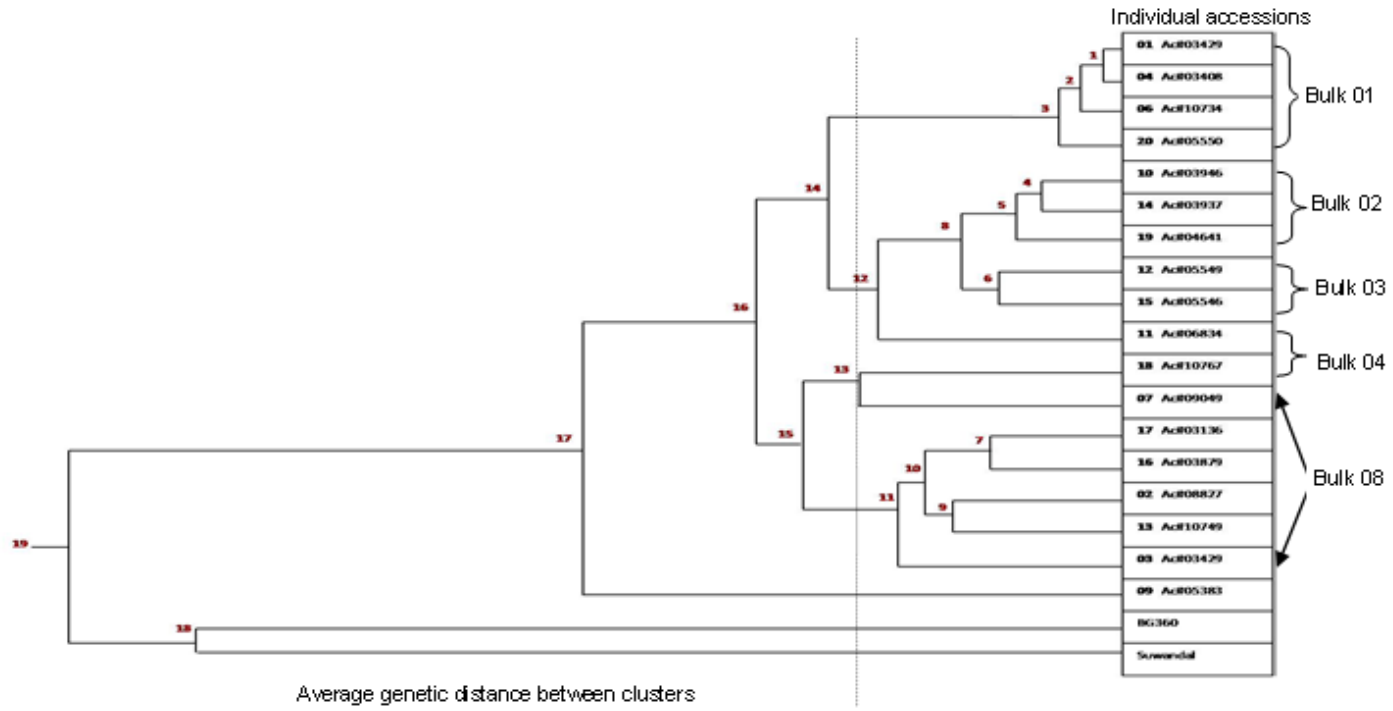



***Figure 5:*** *Genetic Diversity among 20 Individual Accessions based on Analysis of SSR Data Set; Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA, Modified from NEIGHBOR procedure of PHYLIP Version 3.5*

***Table 4:*** *Summary of Heterozygosity Statistics for All Loci; * Expected homozygosty and heterozygosity were computed using Levene (1949), ** Nei's (1973) expected heterozygosity.*

| Locus | Sample Size | Observed Homozygosity | Observed Heterozygosity | Expected Homozygosity* | Expected Heterozygosity* | Nei** | Average Heterozygosity |
|---|---|---|---|---|---|---|---|
| RM560 | 28 | 1.0000 | 0.0000 | 0.5450 | 0.4550 | 0.4388 | 0.0000 |
| RM213 | 28 | 0.9286 | 0.0714 | 0.5741 | 0.4259 | 0.4107 | 0.0357 |
| RM216 | 28 | 1.0000 | 0.0000 | 0.4815 | 0.5185 | 0.5000 | 0.0000 |
| RM270 | 28 | 0.9286 | 0.0714 | 0.4259 | 0.5741 | 0.5536 | 0.0357 |
| RM202 | 28 | 0.9286 | 0.0714 | 0.3889 | 0.6111 | 0.5893 | 0.0357 |
| RM440 | 28 | 0.2857 | 0.7143 | 0.3333 | 0.6667 | 0.6429 | 0.3571 |
| RM571 | 28 | 0.9286 | 0.0714 | 0.5260 | 0.4735 | 0.4566 | 0.0357 |
| RM201 | 28 | 1.0000 | 0.0000 | 0.74 | 0.2646 | 0.2551 | 0.0000 |
| RM217 | 28 | 1.0000 | 0.0000 | 0.7354 | 0.2646 | 0.2551 | 0.0000 |
| RM241 | 28 | 0.9286 | 0.0714 | 0.6799 | 0.3201 | 0.3087 | 0.0357 |
| RM536 | 28 | 0.7857 | 0.2143 | 0.5847 | 0.4153 | 0.4005 | 0.1071 |
| RM412 | 28 | 1.0000 | 0.0000 | 0.7460 | 0.2540 | 0.2449 | 0.0000 |
| RM480 | 28 | 0.9286 | 0.0714 | 0.8016 | 0.1984 | 0.1913 | 0.0357 |
| RM224 | 28 | 1.0000 | 0.0000 | 0.7354 | 0.2646 | 0.2551 | 0.0000 |
| RM259 | 28 | 1.0000 | 0.0000 | 0.6296 | 0.3704 | 0.3571 | 0.0000 |
| RM418 | 28 | 0.8571 | 0.1429 | 0.3095 | 0.6905 | 0.6658 | 0.0714 |
| Mean | 28 | 0.9063 | 0.0937 | 0.5771 | 0.4229 | 0.4078 | 0.0469 |
| St. Dev | | 0.1764 | 0.1764 | 0.1570 | 0.1570 | 0.1514 | 0.0882 |

The summary of heterozygosity statistics for all loci used in the study is shown in table 4. Locus heterozygosity is related to the polymorphic nature or genetic variation of each locus. The observed heterozygosity in the table is defined as the number of individuals heterozygous per locus [26]. High values of heterozygosity reflect high genetic variation of individuals, while low values of heterozygosity reflect less genetically variated homozygous individuals. The highest observed heterozygosity with highest genetic variability is found in RM440 locus with a comparatively high value of 0.7143. Lowest polymorphism or genetic variation (0.0000) among accessions was found out in loci RM560, RM216, RM201, RM217, RM412, RM224 and RM259 from all 16 loci because they were having lowest value for observed heterozygosity or highest value for observed homozygosity. Altogether, considering all loci, the mean value for observed heterozygosity is 0.0937 which concludes rather low level of polymorphism

among accessions for all tested loci. Finally, closely related representative 10 accessions were identified as definite 'Pachchaperumal' Rice accessions, by evaluating both morphological and molecular results obtained. The representative set of 'Pachchaperumal' Rice is comprised of the accessions, 03429, 03408, 10734, 05550, 03946, 03937, 04641, 05549, 05546 and 03136 or 03879 (Duplicates). Focusing on passport data of these accessions, all was received or collected as land races and specially, accessions 5550, 5549 and 5546 are recorded as former Bg accessions (the Rice accessions assessed and improved by Rice Research and Development Institute, Bathalagoda, Sri Lanka). Therefore those 3 accessions can be suggested as most putative 'Pachchaperumal' accessions.

## 4 Conclusion

A significant degree of genetic diversity was found among tested 'Pachchaperumal' Rice accessions in the study. Both clusters based on molecular and morphological descriptors effectively delineated the 20 accessions in fact, molecular clustering more contributively. Eliminating potent morphological off types and bulking accessions based on morphological clusters, then continue molecular analysis bulk wise and lastly proceed with necessary expansions of components, altogether was a promising strategy for this type of a study. The morphological and molecular results were not similar at all and matching to a certain considerable extent. Ten accessions of 'Pachchaperumal Rice were identified as the representative set which are 3429, 3408, 10734, 5550, 3946, 3937, 4641, 5549, 5546, 3136 or 3879. Interestingly, two of them were identified as duplicates for the tested 16 loci (3136 and 3879). Three of these accessions 5550, 5549 and 5546 were identified as more putative accessions as they have been recorded as Bg accessions. These results will contribute to identify the most potential accessions of 'Pachchaperumal' with emphasizing duplicates, out of large number of accessions in the conservation and will help to maintain and exploit most effective accessions in paddy evaluation, breeding programs and seed certification for arising demands.

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