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RESEARCH ARTICLE

Plant Genetics

Genetic diversity analysis of traditional and improved rice genotypes in Sri Lanka using SSR markers

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Abstract: The objective of the present study was to find the genetic diversity of eight improved varieties and eighty-four traditional accessions of rice using nine polymorphic simple sequence repeat (SSR) markers. The SSR markers detected 32 alleles ranging from 2 to 5, with an average of 3.55 alleles per locus, indicating a high genetic diversity. The number of effective alleles (ne) ranged from 1.85 (*RM208*) to 3.84 (*RM493*), with an average of 2.64 alleles per locus, which reconfirms an existing broad genetic diversity. Nei's genetic diversity index was very high (0.5955), indicating a high mean expected heterozygosity (HE). *RM493* recorded the maximum polymorphism information content (PIC) value (0.8814). The mean PIC value was 0.5955 for the used SSR markers. Out of nine SSR markers, seven scored more than 0.5 PIC values, proving their potential to be used as genetic markers. Shannon's information index (I) ranged from 0.65 (*RM208*) to 1.36 (*RM493*), with a mean value of 1.00. All genetic diversity indicators, na, ne, HE, PIC and I reflect the high genetic differentiation in representative sample of rice genotypes. According to the unweighted pair group method with arithmetic mean dendrogram and Nei's genetic distance, the ninety-two rice genotypes were classified into seven groups at an ~85 level of similarity with additional sub-clusters within each group. Rice genotypes with significant genetic divergence can be chosen for upcoming breeding programmes by assessing their positions within the dendrogram. In the dendrogram, genotypes sharing the same name were not clustered together, indicating their distinct genetic backgrounds despite them sharing the same name. The traditional rice accessions clustered together in the dendrogram can be further analysed using more polymorphic SSR markers.

Keywords: Nei's genetic diversity index, polymorphism information content, SSR markers.

INTRODUCTION

The availability of breeding materials with sufficient genetic variation is essential for developing improved new varieties (Temesgen, 2021). Local institutes in several countries, such as the International Rice Research Institute (IRRI), maintain rice gene banks, and IRRI conserves more than 106,800 rice accessions. Germplasm collection, maintenance, conservation, and evaluation are carried out in gene banks (Priyanka *et al.*, 2021). Morphological, physiological, cytological (Jiang, 2013), and molecular marker-based methods are used for diversity analysis in germplasm evaluation (Nakayama, 2005).

Dissection of diversity in genetic materials is essential for plant breeders to select desirable characteristics (Jiang, 2013). Morphological characterization is the simplest method broadly used to analyze germplasm collections (Maji, 2012). Many studies have used phenotypic traits to assess the genetic diversity of rice (Krishnamurthy *et al.*, 2014; Gaballah *et al.*, 2021a). Conventional breeding relies on morphological characterization, though it is time-consuming, labour-consuming, and needs a large population and infrastructure to manage

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the replicated trials. However, phenotypic evaluation may not be accurate due to the environmental effect on phenotype, and molecular-based analysis is required to validate the morphological characterization (Ray *et al*., 2013). Different molecular markers are used for this (Bhanu, 2017). Molecular markers accelerate breeding programmes with greater accuracy (Lema, 2018). Genotypic diversity has been effectively assessed using agronomical traits (Kocaman *et al*., 2020), physiological characteristics (Sanghamitra *et al.*, 2021; Ril *et al*., 2022) and quality characteristics (Utami *et al*., 2017). SSR markers are DNA sequences linked with a specific known location. About 2500 SSR primer pairs were developed for rice (McCouch *et al*., 2002). The copy number changing with the individuals is the source of polymorphism in SSR markers (Schrider & Hahn, 2010). The superiority of SSR markers over other molecular markers is due to their technical simplicity in assessing co-dominant inheritance and the requirement of a small amount of DNA to initiate PCR reactions. Further, SSR markers are relatively low-cost, time-saving, hypervariable, guarantee extensive genomic coverage, and provide high genomic resolution (Ijaz, 2011; Kocaman *et al*., 2020; Ril *et al*., 2022).

The diversity of rice germplasm collections has been studied using SSR markers in different collections: wild rice (Getachew *et al*., 2018; Ngangkham *et al.,* 2019), upland rice (Roy *et al*., 2016), weedy rice (Hanafiah *et al.*, 2022), traditional rice (Dahanayaka *et al.*, 2015; Hemachandra, 2018; Manatunga *et al*., 2018; Weerakoon & Somaratne, 2021; Koodalugodaarachchi *et al*., 2022), *Japonica* and *indica* rice types (Yogi *et al*., 2021), Ethiopian rice genotypes (Getachew *et al*., 2018), Thai rice genotypes (Pathaichindachote *et al*., 2019), and landraces in China (Yang *et al*., 2022).

Sri Lankan traditional rice accessions have been evaluated using SSR markers for different purposes: drought tolerance (Munasunghe *et al*., 2017), development of conventional rice co-collection accessions (Weerakoon & Somaratne, 2021), detection of amylose content (Kottearachchi *et al*., 2014) and flowering time (Ranawake & Mori, 2014).

Several sets of traditional rice accessions with the same names and improved rice varieties were included in the present study. The objectives of the present study were to analyse the genetic diversity among a selection of improved and traditional rice genotypes from Sri Lanka, investigate genetic resemblances among accessions sharing the same name, explore genetic relationships using SSR markers, and identify genetically distant genotypes. The overlap between *Murungakayan3490* accession was found in the present study and study conducted by Manathunga *et al*., (2018). Additionally, a pair of *Kaluheenati* accessions (*5191, 7802*) was previously studied by Siriwardhana *et al*., (2016). No overlapping accessions were identified among the *Suwandal* accessions examined by Gunasena *et al*. (2016), or the *Suduheenati* accessions studied by Tharmarajan *et al*. (2018). The specificity in this study is discrimination between accessions, accuracy of clustering, or phylogenetic analysis and utility in breeding programs.

MATERIALS AND METHODS

Plant materials

Ninety-two rice genotypes, including eighty-four traditional and eight improved varieties of Sri Lankan origin, were collected from the Plant Genetic Resources Centre, Gannoruwa, Sri Lanka (PGRC, 1999). These accessions had been assigned unique accession numbers as part of the documentation process. The farmers used the provided names when referring to them as they were grown in various locations across Sri Lanka. The names/numbers were as follows:
Dahanala5386, Kalubalawee3976, Mawee3618, Ratawee3525, Dahanala6739, Kalubalawee3172, Mawee855, Ratawee3466, Dahanala3917, Kalubalawee3158, Mawee5384, Rathuwee3905, Dahanala3304, Kalubalawee5480, Murungakayan6285, Rathuwee3473, Dikwee4927, Kaluheenati7802, Murungakayan6263, Rathuheenati6249, Dikwee3741, Kaluheenati5191, Murungakayan3921, Rathuheenati5486, Dikwee3504, Kaluheenati4991, Murungakayan3900, Rathuheenati4992, Dikwee3444, Kaluheenati, Murungakayan3809, Suduheenati4932, Dikwee2203, Kaluheenati4621, Murungakayan3495, Suduheenati7799, Elwee3578, Kaluheenati3851, Murungakayan3492, Suduheenati5670, Heenati6402, Kaluheenati3471, Murungakayan3490, Suduheenati3932, Heenati4935, Kuruwee4679, Murungakayan3489, Sudurusamba4362, Heenati4618, Kuruwee3982, PodiweeA8-3109, Sudurusamba3671, Heenati4524, Kuruwee3898, Pokkali3922, Sudurusamba2202, Heenati3998, Kuruwee3552, Pokkali3881, Sudurusamba4363, Heenati3936, Kuruwee3465, Pokkali3701, Heenati3707, Mawee8552, Pokkali3573, Kaluwee4624, Mawee8497, Pokkali3567, Kaluwee3876, Mawee5531, Pokkali3562, Kaluwee3728, Mawee5384, Polayal3661, Kaluwee3212, Mawee4145, Polayal3639, Kalubalawee5481, Mawee3704, Ratawee4580, Kalubalawee5479, Mawee3683, Ratawee3655, Bg250, Bg251, Bg300, Bg350, Bg352, Bg359, Bg360, Bg369

DNA extraction

Dormancy broke (50°C for five days) rice seeds were germinated under dark conditions and grown for two weeks. Genomic DNA was extracted from two weeks old whole seedlings using the cetyl trimethyl ammonium bromide (CTAB) method (Murray & Thompson, 1980).

The concentration of each sample was observed by visual assessment of the band intensity compared to the lambda DNA of known concentrations, using 1% agarose gel. DNA was amplified using nine polymorphic SSR markers (Temnykh *et al*., 2001; McCouch *et al*., 2002) (Table 1).

Table 1: The sequences of the polymorphic SSR primers used for the diversity analysis in rice

Marker	Chromosome no	Forward sequence	Reverse sequence
RM208	2	5′ TCTGCAAGCCTTGTCTGATG 3′	5′ TCTGCAAGCCTTGTCTGATG 3′
RM277	12	5′ CGGTCAAATCATCACCTGAC 3′	5′ CAAGGCTTGCAAGGGAAG
RM248	7	5′ TCCTTGTGAAATCTGGTCCC 3′	5′ GTAGCCTAGCATGGTGCATG
RM464A	9	5′ AACGGGCACATTCTGTCTTC 3′	5′ TGGAAGACCTGATCGTTTCC
RM493	1	5′ TAGCTCCAACAGGATCGACC 3′	5′ GTACGTAAACGCGGAAGGTG
RM589	6	5′ ATCATGGTCGGTGGCTTAAC 3′	5′ CAGGTTCCAACCAGACACTG
RM271	10	5′ TCAGATCTACAATTCCATCC 3′	5′ TCGGTGAGACCTAGAGAGCC
RM122	5	5′ CTTCTTCCGCTTCCTCCCTTCC 3′	5′ TGTACCAGTGCACCGAGAGTTGG 3′
RM280	4	5′ ACACGATCCACTTTGCGC 3′	5′ TGTGTCTTGAGCAGCCAGG 3′

PCR was done in a 20 µL reaction mixture using around 50 ng template DNA, 125 U Taq DNA polymerase, 1.0 mM dNTPs, polymerase buffer (Mg^{2+} plus) (Promega), and ten µM primer in Applied Biosystems GeneAmp 9700 (2012) PCR System (USA). The PCR program was 95°C for 5 min, followed by 25 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 7 min. The PCR products were run on a 1.0% poly acrylamide gel using 1x TBE buffer. Gel plates were silver stained for visualization. Banding profiles generated by each set of markers were compiled into a co-dominant data matrix by manual scoring according to Wu & Tanksley (1993). Tabulated scored data were analyzed using Popgene 1.31 (Yeh *et al*., 1999).

RESULTS AND DISCUSSION

Applying SSR markers in high-resolution genetic studies is critical for obtaining insights into the genome-wide distribution of genetic variation. Though using a limited set of nine polymorphic SSR markers may not directly elaborate the genetic parameters extensively, the derived values and advanced analytical methods can facilitate a nuanced comprehension of the underlying genetic dynamics.

Allelic polymorphism (na)

Allelic polymorphism is defined as the existence of more than one allele at a locus at a frequency greater than 5% in the population (Nei, 1984). SSR markers are highly polymorphic, even in closely related genotypes (Ellis & Burke, 2007). In the present study, nine polymorphic SSR markers identified thirty-two alleles across ninety-two genotypes. Out of nine polymorphic alleles, the maximum number of alleles (5) was generated by *RM277* and *RM464A*, followed by *RM493*, *RM589*, and *RM280* (4 alleles each). The minimum number of alleles was generated by *RM208* and *RM248* (3 alleles each). The average number of alleles per polymorphic locus was 3.55 (Table 2).

A study that used 41 rice genotypes and 15 SSR markers reported 68 total alleles, and the number of alleles detected by a marker ranged from 2 to 8 (Freeg & Anis, 2016). The number of alleles per locus ranged from 2 to 5 with an average value of 3.7 in the study carried out by Tripathi *et al*. (2020), and the average number of alleles per locus ranged from 1.20 to 4.30 with an average value of 2.45 according to Gaballah *et al*. (2021a). Similar results were also reported by Donde *et al.* (2019), who investigated the genetic variability of sixteen rice landraces using sixty-three primers where alleles per locus ranged from 1 to 3 with an average value of 1.5. Allelic polymorphism ranged from 2 to 47 alleles per locus in different studies that used SSR markers in rice diversity analysis (Table 2).

Table 2: Genetic parameters as revealed by polymorphic SSR markers

Locus	na	ne	Nei	HE	PIC	I
RM208	2.0000	1.8506	0.4596	0.4623	0.4279	0.6522
RM277	5.0000	3.3741	0.7036	0.7090	0.5346	1.3413
RM248	2.0000	1.9656	0.4912	0.4949	0.3489	0.6844
RM464A	5.0000	2.2484	0.5552	0.5594	0.7076	0.9306
RM493	4.0000	3.8352	0.7393	0.7444	0.8814	1.3651
RM589	4.0000	3.3153	0.6984	0.7039	0.7448	1.2777
RM271	3.0000	2.0436	0.5107	0.5135	0.5108	0.7462
RM122	3.0000	2.1851	0.5424	0.5465	0.5444	0.8465
RM280	4.0000	2.9310	0.6588	0.6639	0.6589	1.1617
Mean	3.5556	2.6388	0.5955	0.5997	0.5955	1.0006
SD	1.1304	0.7333	0.1048	0.1058	0.1670	0.2886

na = observed number of alleles, ne = effective number of alleles, HE = expected heterozygosity computed using Levene (1949), Nei = Nei's (1973) gene diversity, PIC = polymorphism information content

Nei's (1973) expected heterozygosity, I = Shannon's Information index [Lewontin (1972)]

The number of effective alleles (ne)

The number of effective alleles (ne) is the number of alleles that can exist in a population (IPGRI and Cornell University, 2003). The number of effective alleles per locus ranged from 1.8506 (*RM208*) to 3.8352 (*RM493*), with an average of 2.6388 in the present study (Table 2). In contrast to the effective allele count presented by Getachew *et al*. (2018), which was derived from an analysis of 426 distinct genotypes (Table 4), the findings of the present study highlight a notably higher level of genetic diversity within the examined rice genotypes (*Supplementary table 1*). The number of effective alleles in the present study was greater compared to that reported by Getachew *et al*. (2018) which used 426 different genotypes (Table 4), which indicates an existing greater genetic diversity in the studied rice genotypes.

Nei's genetic diversity

Nei's genetic diversity shows the genetic variance within specific populations (Nei, 1973). In the present study, Nei's genetic diversity ranged from 0.4596 to 0.7393, indicating a significant genetic variation among genotypes. The recorded Nei's genetic diversity index is more remarkable than reported in previous studies (Jasim *et al*., 2018; Dao *et al*., 2018; Verma *et al*., 2019; Razak *et al*., 2020) and less than the Nei's genetic diversity index reported by Sarif . (2020). The maximum Nei's genetic index (0.7393) recorded in the present study is almost similar to the reported value of 0.744 by Koodalugodaarachchi *et al.* (2022) for Sri Lankan rice genotypes, while Dahanayaka *et al*. (2015) reported 0.8078 as the maximum Nei's genetic index for a separate Sri Lankan rice genotype.

Expected heterozygosity (HE)

The expected heterozygosity (HE) is the probability that two alleles chosen randomly from a population differ (Nei, 1973). RM493 recorded the highest HE value (0.7444), and the average HE value was 0.5997, emphasizing the heterozygous nature of the studied genotypes. The HE values ranged from 0 to 0.94 in different diversity studies in rice (Supplementary Table 1). Compared to most of them, 0.7444 is a higher HE value for diversity analysis in rice.

Polymorphism information content (PIC)

PIC processes the ability of a marker to identify polymorphisms and therefore decides the utility of the specific SSR marker as a suitable genetic marker (Serrote *et al*., 2020). Other than *RM208* and *RM248*, all seven remaining markers showed more than 0.5 PIC values, suggesting they could be used as genetic markers. Markers with high PIC values indicate greater genetic diversity within the population for those alleles. Genetic diversity is essential for plant breeding programmes as it provides a broader range of traits and characteristics that breeders can select from to develop improved cultivars. Further, higher PIC values suggest that the markers capture variations in traits that interest breeders, such as disease resistance, yield, quality, or environmental adaptability. These markers could lead to the identification of plants with desirable traits.

Moreover, markers with high PIC values are more informative, as they can distinguish between many different genotypes. This efficiency allows breeders to make more accurate selections in a shorter period. When markers have higher PIC values, they are more likely to be linked to genes that significantly impact the traits of interest. This means that selecting plants based on these markers has a higher chance of resulting in the desired traits in the next generation. Markers with higher PIC values are more likely to be spread across the genome, reducing the likelihood of "linkage drag," where unwanted traits are inadvertently transferred along with the desired trait during breeding. *RM493* and *RM248* reported the maximum (0.8814) and minimum (0.3489) PIC values, respectively, and the average PIC value was 0.5955. Different ranges of PIC values have been recorded, such as 0.4824–0.8078 (Dahanayaka *et al.*, 2015), 0.36–0.98 (Kumar *et al.*, 2012), 0.41–0.89 (Hue *et al*., 2018) in rice diversity analysis studies, indicating a long history of domestication leading to trait evolution and selection.

Shannon's Information Index (I)

Shannon's Information index is a mathematical explanation of originally developed species diversity in a particular community based on the number of species present and individuals per species. The increasing number of species results in higher diversity. When Shannon's diversity index was applied to the diversity of the rice genotypes in a study, the values ranged from 0 to 5, and 1.5 to 3.5 is the typical range (CNR-University of Idaho, 2009). Shannon's index in the present study ranged from 0.6522 *(RM208*) to 1.3653 (*RM493*), averaging 1.0006. The average of Shannon's indices of two previous studies were reported as 0.58 (Jasim *et al*., 2018) and 2.8 (Sarif *et al*., 2020). The comparison of the genetic parameters of the present study with other similar studies carried out on rice in the near past shows the broad genetic diversity of Sri Lankan rice genotypes (*Supplementary table 1*).

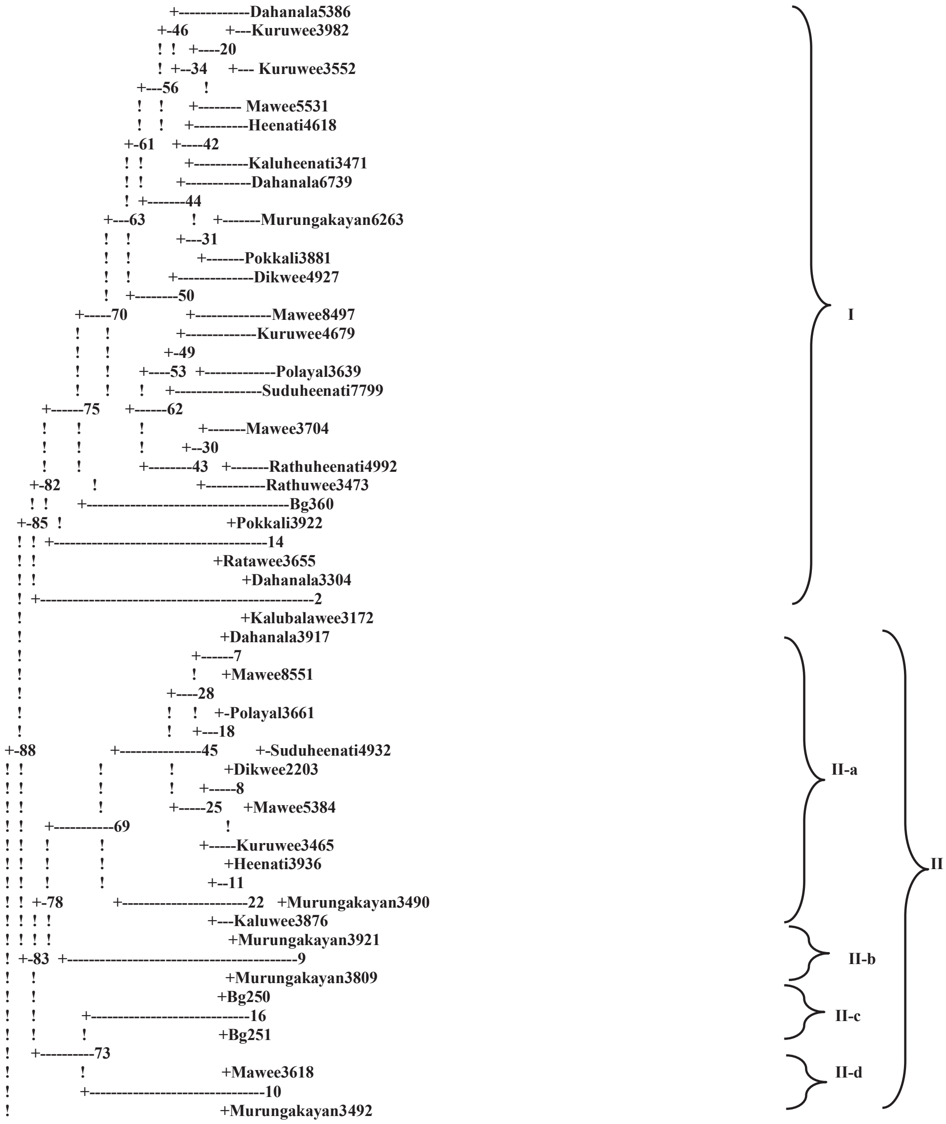
Analysis of genetic relationships among ninety-two Sri Lankan rice genotypes

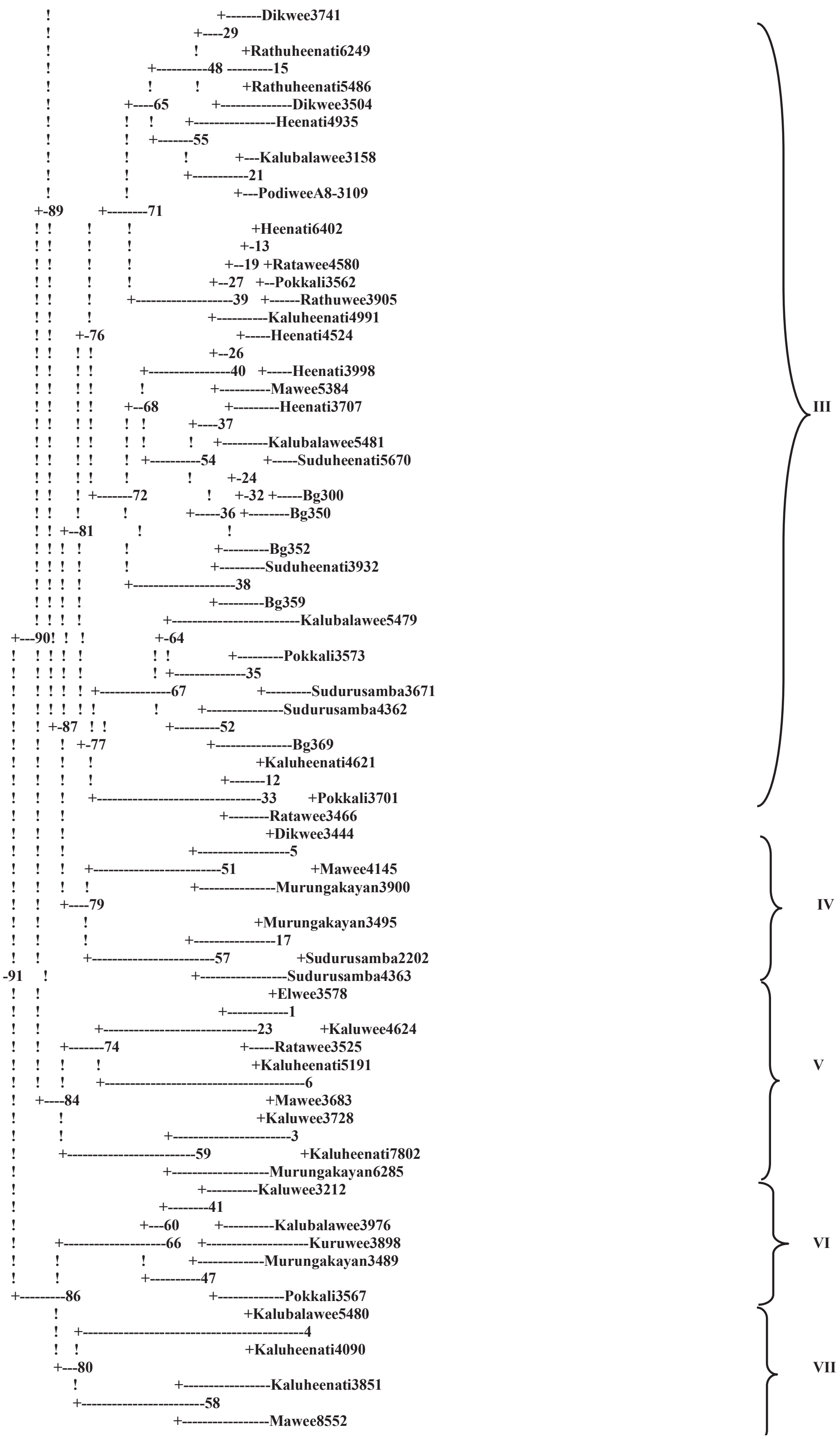
Using Nei's genetic distance and the UPGMA method of clustering, ninety-four rice genotypes were clustered into seven distinct groups at 85% similarity (Nei's genetic distance) (Figure 1). Nei's genetic identity was automatically calculated based on bootstrap analysis in Popgene. In the UPGMA dendrogram, a group I consisted of 22 rice genotypes, including one improved rice variety, *Bg360*. Among traditional accessions in group I, there were three *Dahanala (5386, 6739, 3304*), three *Kuruwee (3982, 3552, 4679**)**,* three *Mawee (5531, 8497, 3704),* and two *Pokkali (3881, 3922)* accessions. The other accessions in group I were *Heenati4618, Kaluheenati3471, Murungakayan6263, Dikwee4927, Polayal3639, Suduheenati7799, Rathuheenati4992, Rathuwee3473, Ratawee3655,* and *Kalubalawee3172*. Group one was divided into four subgroups at the Nei's genetic distance of 74%, and improved variety *Bg360* was separated from the other traditional rice accessions to make a separate subgroup. The largest subgroup of group I consisted of seventeen traditional rice accessions, with three *Mawee* and three *Kuruwee* rice accessions. In this group, most genotypes take more than 100 days for flowering, including improved variety *Bg360* (*unpublished data*).

Group II contained sixteen genotypes including improved varieties *Bg250* and *Bg251.* It was divided into four subgroups at the Nei's genetic distance of 70%. Group II-a had ten different traditional rice accessions. Group II-b consisted of two *Murungakayan* accessions (*3921, 3809*), and group II-c was separated with two improved rice varieties, *Bg250* and *Bg251.* Manathunga *et al*. (2018) identified *Murungakayan3490* as a representative accession, among others, namely *5378, 5614, 6279, 12824, 3329, 3491*, and *5611*, that possessed true-to-type characteristics in their study. Among these accessions, only *Murungakayan3490* was studied in the present research. The present results revealed that *Murungakayan3490* clustered with *Murungakayan3921* and *Murungakayan3809*, demonstrating their genetic similarities (Figure 1). Group III was the largest group, containing thirty-one rice genotypes. Five improved rice varieties (*Bg300, Bg350, Bg352, Bg359,* and *Bg369*) out of eight were clustered in this group. In addition, some traditional rice accessions with the same name, *i.e.*, *Rathuheenati (6249, 5486), Dikwee (3504, 3741), Pokkali (3562, 3573, 3701), Heenati (4524, 3998, 6402, 4935, 3707), Kalubalawee (5481, 5479, 3158), Suduheenati (5670, 3932), Sudurusamba (3671, 4362), Kaluheenati (4621, 4991),* and *Ratawee (4580, 3466)* were in group III.

Group IV comprised two *Murungakayan (3900, 3495)* and two *Sudurusamba (2202, 4363)* accessions. Group V contained eight traditional rice accessions: *Kaluwee (4624, 3728), Kaluheenati (5191, 7802), Mawee3683, Murungakayan6285, Ratawee3525,* and *Elwee3578.* The study of Siriwardhana *et al*. *(2016)* has reported nine genetically related accessions *(4089, 5191, 5385, 7802, 4087, 6713, 12932, 12926,* and *4091)* as a representative set for the pure line of *Kaluheenati.* Interestingly, the present study confirmed the genetic similarities of the Kaluheenati 5191 and 7802 accessions by clustering them together in Group V.

Group VI was the smallest group with four traditional rice accessions, including *Kaluheenati (4090, 3851), Mawee8552, and Kalubalawee5480*.





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      !                       +-------Dikwee3741
      !                    +----29
      !                    !      +Rathuheenati6249
      !              +----------48 ---------15
      !              !     !      +Rathuheenati5486
      !          +----65      +-------------Dikwee3504
      !          !   !    +---------------Heenati4935
      !          !   +-------55
      !          !        !       +---Kalubalawee3158
      !          !        +----------21
      !          !                +---PodiweeA8-3109
    +-89     +--------71
     ! !     !    !                    +Heenati6402
     ! !     !    !                  +-13
     ! !     !    !                +--19 +Ratawee4580
     ! !     !    !              +--27   +--Pokkali3562
     ! !     !    +-----------------39   +------Rathuwee3905
     ! !     !                   +---------Kaluheenati4991
     ! !   +-76                     +-----Heenati4524
     ! !    ! !                  +--26
     ! !    ! !       +---------------40   +-----Heenati3998
     ! !    ! !       !          +---------Mawee5384
     ! !    ! !    +--68            +---------Heenati3707
     ! !    ! !    !  !      +----37
     ! !    ! !    !  !      !   +---------Kalubalawee5481
     ! !    ! !    !  +----------54      +-----Suduheenati5670
     ! !    ! !    !         !      +-24
     ! !    ! +-------72        !    +-32 +-----Bg300
     ! !    !      !         +-----36 +--------Bg350
     ! ! +--81       !                !
     ! ! !  !      !                +---------Bg352
     ! ! !  !      !               +---------Suduheenati3932
     ! ! !  !      +-------------------38
     ! ! !  !                      +---------Bg359
     ! ! !  !             +-----------------------Kalubalawee5479
 +---90! !  !            +-64
 !   ! ! !  !            ! !             +---------Pokkali3573
 !   ! ! !  !            ! +-------------35
 !   ! ! !  ! +-------------67               +---------Sudurusamba3671
 !   ! ! !  ! !          !       +---------------Sudurusamba4362
 !   ! +-87  ! !            +---------52
 !   !   !  +-77                 +---------------Bg369
 !   !   !   !                          +Kaluheenati4621
 !   !   !   !                    +-------12
 !   !   !   +--------------------------------33       +Pokkali3701
 !   !   !                        +--------Ratawee3466
 !   !   !                                   +Dikwee3444
 !   !   !                   +-----------------5
 !   !   !  +-----------------------51                +Mawee4145
 !   !   !   !                +--------------Murungakayan3900
 !   !   +----79
 !   !      !                            +Murungakayan3495
 !   !      !               +---------------17
 !   !      +---------------------57              +Sudurusamba2202
-91    !                      +----------------Sudurusamba4363
 !   !                                      +Elwee3578
 !   !                             +-----------1
 !   !        +----------------------------23         +Kaluwee4624
 !   !   +-------74                    +-----Ratawee3525
 !   !   !      !                        +Kaluheenati5191
 !   !   !      +---------------------------------------6
 !   +----84                                   +Mawee3683
 !       !                               +Kaluwee3728
 !       !             +---------------------3
 !       +-----------------------59                  +Kaluheenati7802
 !                     +------------------Murungakayan6285
 !                          +----------Kaluwee3212
 !                     +--------41
 !                  +---60       +----------Kalubalawee3976
 !      +-------------------66    +-------------------Kuruwee3898
 !      !            !        +-------------Murungakayan3489
 !      !            +----------47
 +---------86                     +-------------Pokkali3567
        !                                 +Kalubalawee5480
        !  +----------------------------------------4
        !  !                              +Kaluheenati4090
        +---80
           !              +-----------------Kaluheenati3851
           +------------------------58
                          +-----------------Mawee8552
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Figure 1: UPGMA Dendrogram based on Nei’s (1978) genetic distance among ninety-two rice genotypes

A similar result has been reported in a diversity analysis where a UPGMA dendrogram of 24 rice cultivars based on a combination of SSR and sequence-tagged site (STS) markers resulted in five clusters at a genetic similarity coefficient value of 0.84 (Mosab-bin *et al.*, 2022). Another study that analysed thirty genotypes using thirty-five SSR markers resulted in five distinct clusters at a genetic similarity level of 66% (Seetharam *et al.*, 2009), whose cluster diversity is narrower than the present study's diversity. Nine clusters were reported in a study that used sixty-two rice genotypes with a comparatively low dissimilarity coefficient of 0.40 in Malaysia (Anisuzzaman *et al*., 2022). Despite using 151 SSR markers for 24 Philippine rice genotypes, the dendrogram created three distinct clusters at a 40% similarity level (Lapitan *et al*., 2007). Two *Kaluheenati* accessions *(4090, 3851)* were clustered in a previous diversity analysis using SSR markers (Siriwardana *et al*., 2016). The genetic diversity of Sri Lankan traditional rice accessions with the same name, including *Suwandal* (Gunasena *et al*., 2016), *Murungakayan (*Manathunga *et al*., *2018), Kaluheenati* (Siriwardhana *et al*., 2016), and *Suduheenati* (Tharmarajan *et al*., 2018) was studied using SSR markers. According to Gunasena *et al*. (2016), there were no duplicate accessions in the studied *Suwandal* accessions. Manathunga *et al*. (2018) revealed four sets of *Murungakayan* duplicates, and Siriwardhana *et al*. (2016) identified nine true-to-type *Kaluheenati* accessions. Out of nineteen *Suduheenati* accessions, nine accessions were identified as representatives of *Suduheenati* accessions by Tharmarajan *et al*. (2018).

The well-spanned dendrogram constructed using the UPGMA method and Nei's genetic distance emphasizes the usefulness of SSR markers dissecting genetic diversity in rice. Accessions clustered into one group are supposed to have high genetic similarity, while those clustered away from others are considered divergent.

The dendrogram helps assess the genetic diversity present in different rice germplasm collections. This information is essential for identifying unique and diverse genetic resources that can be conserved to ensure a wide range of traits and adaptations for future breeding efforts.

SSR markers allow for accurate and reliable characterization of rice germplasm. They help distinguish between different rice genotypes, ensuring that the correct genetic material is conserved. Further, the dendrogram depicts the genetic relationships among different rice genotypes. This information guides the selection of diverse parents for breeding programs and assists in avoiding redundancy in conservation efforts. Future repetition or regular monitoring of rice germplasm using SSR markers helps detect any loss of genetic diversity over time for identifying vulnerable or endangered accessions that require immediate conservation actions.

Table 5: Cluster groups of rice genotypes with the same name by UPGMA Dendrogram

Group	Traditional rice genotypes	Improved rice accessions
I	*Dahanala5386, Dahanala6739, Dahanala3304, Kuruwee3982, Kuruwee3552, Kuruwee4679, Mawee5531, Mawee8497, Mawee3704, Pokkali3881, Pokkali3922*	*Bg360*
II	*Mawee8551, Mawee3618, Mawee5384, Murungakayan3921, Murungakayan3809, Murungakayan3490, Murungakayan3492*	*Bg250, Bg251*
III	*Rathuheenati6249, Rathuheenati5486, Dikwee3504, Dikwee3741, Pokkali3562, Pokkali3573, Pokkali3701, Heenati4524, Heenati3998, Heenati6402, Heenati4935, Heenati3707, Kalubalawee5481, Kalubalawee5479, Kalubalawee3158, Suduheenati5670, Suduheenati3932, Sudurusamba3671, Sudurusamba4362, Kaluheenati4621, Kaluheenati4991, Ratawee4580, Ratawee3466*	*Bg300, Bg350, Bg352, Bg359, Bg369*
IV	*Murungakayan3900, Murungakayan3495, Sudurusamba2202, Sudurusamba4363*	
V	*Kaluwee4624, Kaluwee3728, Kaluheenati5191, Kaluheenati7802*	
VI	*Kaluwee3212, Kalubalawee3976,*	
VII	*Kaluheenati4090, Kaluheenati3851, Kalubalawee5480*	

Though the outcome of the present study is limited due to the small number of SSR markers used, the output of this type of study facilitates the exchange of rice germplasm between different gene banks and research institutions; accurate genetic characterization ensures that exchanged materials are true to their original identity and maintain their genetic integrity. Furthermore, genotype position in the dendrogram can be considered for selecting appropriate parental lines for rice breeding programs, promoting the incorporation of diverse and desirable traits into new varieties while avoiding genetic bottlenecks.

Clustering traditional rice accessions with the same name but different accession numbers into one cluster gives evidence to consider them as duplicated collections (Table 5). However, the strength of conclusions drawn from diversity analysis studies regarding genetic similarities and dissimilarities is positively correlated with the increased number of SSR markers that span across all twelve chromosomes in rice.

CONCLUSIONS

The present study revealed a broad genetic diversity in ninety-two rice accessions. SSR markers found high genetic diversity with an average of 3.55 alleles per locus, effective alleles ranged from 1.85 to 3.84 per locus, and Nei's genetic diversity index was 0.5955. *RM493* had the highest PIC value (0.8814) among the markers used. *RM 277, RM464A, RM473, RM589, RM271, RM122*, and *RM280* SSR markers that scored more than 0.5 PIC values are promising genetic markers for future breeding programmes. Rice varieties exhibiting a significant genetic variation may be selected for forthcoming breeding endeavours by evaluating their placement within the dendrogram. This study confirmed the presence of genetically distinct genotypes among various accessions sharing identical names (*e.g.*, *Murungakayan, Mawee, Kaluwee, Sudurusamba, Kalubalawee,* etc.). Increasing the number of polymorphic SSR markers in future studies will effectively address the unsolved genetic dissimilarities among rice genotypes clustered together at lower cluster distances. Such studies will contribute to establishing a co-collection within Sri Lanka's traditional rice germplasm.

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Supplementary Table 1: Comparison of the genetic parameters of the present study with other similar studies carried out in rice

Genotypes	Markers	Allele range /locus	Total na	ne	Nei	HE	PIC	I	Reference
92	9	2 to 5 (Avg 3.555)	32	1.85 to 3.83 (Avg 2.64)	0.4596 to 0.7393 (Avg 0.5955)	0.4623 to 0.7444 (Avg 0.5997)	0.3489 to 0.8814 (Avg 0.5955)	0.65 to1.27 (Avg 1.00)	Present study
32	31	6 to 0.303	124		0.169- 0.744		0-161-0.697		(Koodalugodaarachchi *et al.*, 2022)
32	6	3 to 7	23				0.4824-0.8078		(Dahanayaka *et al.*, 2015)
54	59	2 to 9 (Avg 4.24)	250		0.4325		0.0688-0.8854 (Avg 0.3940)		(Dao *et al.*, 2018)
27	12	3 to 5 (Avg 3.7)	40				0.38-0.65 (Avg 0.56)		(Tripathi *et al.*, 2020)
32	34	11 to 38 (Avg 20.76)	706		28.17	0.94	0.86-0.97 (Avg 0.92)	2.8	(Sarif *et al.*, 2020)
46	1536 SNP	1.944 to 2.000			0.268		0.173-0.259 (Avg 0.213)		(Razak *et al.*, 2020)
64	20	1 to 10	467				0.36-0.98		(Kumar *et al.*, 2012)
5	9		74						(Sari *et al.*, 2021)
462	264	2 to 47 (Avg 12.88)	3361				0-0.93		(Chen *et al.*, 2021)
15	10	2 to 13 (Avg 7.20)	72	1.20 to 4.30 (Avg 2.45)		0.94-1.00 (Avg 0.98)	0.83-0.99 (Avg 0.94)		(Gaballah *et al.*, 2021a)
60	50	6, 2.	279	23 (Avg 2.3)					(Beşer and Mutafçilar, 2020)
30	10	28 (Avg 2.8)		23 (Avg 2.21)			0.60-0.081 (Avg 0.42)		(Farahzadi *et al.*, 2020)
426	67	2 to 19 (Avg 6.49)	440	0.85 - 2.3 (Avg 1.54)		0.06-0.48 (Avg 0.27)	0.09-0.72		(Getachew *et al.*, 2018)
90	40	3 to 12 (Avg 7.1)	184				0.41-0.89 (Avg 0.74)		(Hue *et a*l., 2018)
50	32			0.36	0.05-0.98 (Avg 0.36)	0.60	0.25-0.98 (Avg 64)	0.22-0.91 (Avg 0.58)	(Jasim Aljumaili *et al.*, 2018)
130	19	2.6	50			0.0-0.767	0.0-0.718		(Mishra *et al.*, 2018)
114	65	2.26	147		0.118-0658 (Avg 0.334)		0.51		(Verma *et al.*, 2019)
16	63	1-3 (Avg 1.5)							(Donde *et al.*, 2019)

na = observed number of alleles, ne = effective number of alleles, HE = expected heterozygosity computed using Levene (1949), Nei = Nei's (1973) genetic diversity, PIC = polymorphism information content