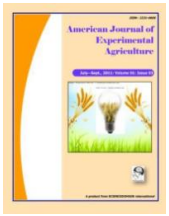

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# Assessment of Genetic Diversity of Elite Indian Rice Varieties Using Agro-Morphological Traits and SSR Markers

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***Authors' contributions***

*Authors KK, KV, RMS designed the experiments. Author KK performed most of the experiments. Authors RMS, RS and PR reviewed experimental design and contributed in experiments and all draft of the manuscript. Author KK, SM, LVS and SRC managed analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

Assessment of genetic variability of the crop varieties is essential to assure selection of genetically divergent lines useful for the future breeding programmes. Thus, genetic diversity assessment of 24 elite Indian rice varieties was performed based on 24 agro-morphological traits and 86 SSR markers. The morphological and grain traits exhibiting significant variation are useful for discrimination of the rice varieties and were confirmed by Principal Component Analysis. Genetic

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diversity assessment based on SSR markers displayed genetic similarity coefficients and grouped the varieties into five major clusters. The genetic population structure obtained was predominantly associated with UPGMA clustering and the structure bar plot. Cluster analysis based on both phenotype and SSR marker data did not show perfect congruence between the two measures of genetic diversity. However, the correlation between morphological and molecular diversity was positive and significant ($r = 0.36$ $P<10^{-4}$) indicating usefulness in classification of rice varieties.

*Keywords: Genetic diversity; agro-morphological; characterization; Indian varieties; simple sequence repeats; Oryza sativa.*

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops that provide food for more than half of the world population. It plays a vital role in the national food security in India and contributes a major source of calories for the urban and rural populace [1]. With changing consumer preferences and the demand by the export market, rice breeding program in India has received significant attention with respect to improvement of yield with grain quality. In spite of more than 900 rice varieties have been developed in India, only considerable number of elite rice varieties have been released to suit different agro-climatic conditions which includes in the present study contributing ~ 29% of the total rice production in the country and being cultivated in many rice growing states (http://agricoop.nic.in). In order to produce improved varieties in future, assessment of the variability among these elite rice varieties is also essential which will enable the identification of genetically divergent varieties useful in future breeding programmes.

Characterization and genetic diversity of rice germplasm is well progressing globally, nevertheless, knowledge regarding the amount of phenotypic and genotypic variation specific to the geographical region which will enable to design effective breeding programs in order to achieve high productivity. Further, it has a great importance for seed production, certification and cultivar propagation and implementation of Protection of Plant Varieties and Farmer's Right Act of India (http://www.planthauthority.org). Traditionally, morphological traits have been used to evaluate distinctness and to establish broad description of rice varieties [2,3]. However, despite the fact that the varieties growing in different agro-climatic regions differ substantially from each other in agronomical traits, they are difficult to characterize using only morphological characteristics recommended by the International Union for the Protection of New Varieties of Plants (UPOV) descriptors [4]. Moreover, rice breeding tends to produce elite varieties which are phenotypically less distinct due to narrow genetic base of the populations and in such situations morphological traits seems inadequate because of environmental influence [2]. Besides, morphological variation does not always reflect real genetic variation because of genotype x environment interactions and the largely unknown genetic control of polygenic morphological and agronomic traits [5].

Molecular marker based approaches provide an opportunity to look into the genotypic basis of the observed phenotypic variation in released varieties [6]. Different types of molecular markers such as RAPDs [7,8], RFLPs [9], AFLPs [10] and ISSRs [7,11] have been used for estimation of genetic diversity and development of molecular fingerprints in rice. Among these, simple sequence repeats (SSRs) or microsatellites are the most popular markers in rice research [12]. SSR markers are becoming markers of choice for molecular characterization and genetic diversity studies in rice [13,14]. Breeding of rice varieties in India has been developed based on few important traits, mainly plant height, flowering, pest and disease resistance, maturity and yield. So far, no comprehensive study based on molecular marker in particular has been documented in the existing popular rice varieties. In view of the above, we attempt to characterize and estimate the genetic diversity in elite rice varieties grown in India using agro-morpho traits and SSR markers along with a correlation between the two diversity estimates.

## 2. MATERIALS AND METHODS

### 2.1 Plant materials

Pure seeds (breeder seed) of 24 elite rice varieties collected from the Acharya N G Ranga Agricultural University, Hyderabad, Tamil Nadu

Agricultural University, Coimbatore and Directorate of Rice Research, Hyderabad, TS, India were used for the present study (Table 1).

## 2.2 Agro-morphological Characterization

The field experiments were carried out in three replications (5×6 m plot size with a spacing of 30 ×20 cm) and all the cultivars were grown under screen-house simultaneously to confirm qualitative traits at Directorate of Rice Research, Hyderabad, India during dry season 2011-12 and 2012-13 for recording stable and uniform morphological characteristics. Ten plants were selected at random and tagged in each variety per replication. The highly variable plant morphological and grain traits in rice cultivars were selected on the basis of variability among closely related varieties [15]. A set of 16 key plant morphological traits, which included qualitative and quantitative characteristics and their states (scores) were recorded as per the National/Indian guidelines for the conduct of tests for Distinctness, Uniformity and Stability (DUS) (http://www.plantauthority.gov.in). The data on quantitative characteristics recorded for two seasons were grouped and the average values were used for statistical analysis. The grain quantitative and qualitative traits namely grain weight, grain length and width, decorticated grain length and width, shape of the de-husked grain, amylose content of endosperm (%) and gelatinization temperature was determined and grouped as per DUS testing protocol (http://www.plantauthority.gov.in).

## 2.3 SSR Marker Analysis

Total genomic DNA was isolated from 10-15 day old seedlings as per the protocol of Zheng et al. [16]. A total of 86 hyper-variable SSR (microsatellite) primer pairs purchased from Integrated DNA Technologies, USA were used for PCR amplification. The SSR markers were selected based on their polymorphism content and distribution across the 12 rice chromosomes. PCR was carried out in 10 µl reaction containing 0.2 µM of each primer, 200 µM of dNTPs, 1X PCR assay buffer (50 mM KCl,10 mM, Tris HCl ($P^H$ 8.3), 1.5 mM of $MgCl_2$, 0.1% gelatin, 15 ng of DNA and 0.5 unit of *Taq* DNA polymerase. The amplification was performed with initial denaturation at 94ºC for 5 min followed by 35 cycles of 94ºC for 30 sec, 55ºC for 1 min, 72ºC for 7 minutes before cooling at 4ºC. The PCR products were electrophoretically resolved on 3% superfine grade agarose (Amresco, USA) stained with ethidium bromide and visualized under UV.

**Table 1. Details of 24 elite Indian rice varieties used in the study**

| Variety | Year of release | Parentage |
|---|---|---|
| ADT 43 | 1998 | IR 50 x White ponni |
| ASD 19 | 1995 | Lalnakanda x IR 30 |
| BPT 5204 (Samba mahsuri) | 1986 | GEB 24 x TN 1 x Mahsuri |
| GEB 24 | 1929 | Mutant of Konmani |
| IR 64 | 1992 | IR 5657-33-2-1 x IR 2061-465-1-5-5 |
| JGL 384 (Polasa prabha ) | 2002 | WGL 48684 x BPT 5204 |
| JGL 1798 (Jagtial sannalu ) | 2002 | BPT 5204 x WGL 48684 |
| Mahsuri | 1972 | 65 x Mayang Ebos 6080 x 2 |
| MTU 1001 ( Vijetha ) | 1995 | MTU 5249 x MTU 7014 |
| MTU 1010 (Cottondora sannalu) | 2000 | MTU 2067 x IR 64 |
| MTU 2067 (Chaitanya ) | 1988 | Sowbhagya x ARC 5984 |
| MTU 2077 (Krishnaveni) | 1989 | Sowbhagya x ARC 5984 |
| MTU 4870 (Deepthi ) | 2000 | MTU 4569 x ARC 6650 |
| MTU 5249 (Vajram) | 1986 | Sowbhagya x ARC 6650 |
| MTU 7029 (Swarna) | 1982 | Vasishtha x Mahsuri |
| RDR 7555(Rudrama) | 1991 | HR 19 x TN 1 |
| RDR 763 (Indur samba) | 1997 | BPT 5204 x WGL 13400 |
| WGL 14 (Warangal sannalu ) | - | BPT 5204 x ARC 5984 / BPT 3291 |
| WGL 13400 (Surekha) | 1976 | IR-8 x Siam 29 |
| WGL 20471 (Erra mallelu ) | 1991 | BC 5-55 x W 12708 |
| RPW6-17 (Phalguna) | 1977 | IR-8 / Siam 29 |
| RNR 23064 (Taramathi) | - | BPT 5204 x Tellahamsa |
| Tellahamsa | 1971 | HR 12 x TN 1 |
| Kalajeera | - | Land race |

## 2.4 Data Analysis

Univariate descriptive statistical data for quantitative traits were computed for mean, range, coefficient of variation and standard deviation. Before analysis, the data was standardized by subtracting the mean value and dividing it by the standard deviation. Principal Component Analysis (PCA) was done on observed morpho-grain traits with the standardized data. Euclidean distances were calculated for all the traits considered together, morphological traits and for the SSR. Each dataset was plotted in a dendrogram using the average linkage method cluster analysis across varieties. With the molecular marker data, Jaccard's index was calculated for genetic distance among the varieties to generate a dendrogram. These analyses were performed in R 2.9.1, using the software packages Vegan 1.15-4 [17] and the base package Stats. Only clear and unambiguous SSR amplicons were scored for their presence and absence among the cultivars as '1' and '0', respectively. The binary data matrix was subjected to further analysis. Sequential Agglomerative Hierarchial Non overlapping (SAHN) clustering was done on the squared Euclidean distance matrix and the similarity matrix was generated based on Jaccard's index by utilizing the UPGMA algorithm through the NTSYS pc 2.0 software [18]. Polymorphism Information Content (PIC) for each SSR was calculated according to the formula: PIC =1- ( $\sum$ piY) while '1' is the total number of alleles detected for SSR marker and 'pi' is the frequency of the $i^{th}$ allele [19]. All computations were performed with appropriate procedures of the NTSYS-pc version 2.1 software [18].

The genetic structure in the elite Indian rice varieties was inferred using the software Structure 2.3.2 [20] based on the amplification pattern of the SSR markers. Structure employs a model based clustering method that accounts for Hardy-Weinberg and linkage disequilibrium and attempts to find population groupings that minimizes linkage disequilibrium. Markers used in this study were distributed across the rice genome and can therefore be assumed to be independent. To estimate the number of sub-populations (the K parameter), the dataset was analyzed allowing the value of K ranging from 1 to 5. Five independent runs were carried out for each K value. All the parameters were set to their default values. An admixture model was chosen as it can account for some individuals having mixed ancestry. The optimal value for number of sub-populations was determined based on likelihood variance values obtained for each run using $\Delta$K, an *ad-hoc* parameter described by Ewens et al. [21]. A bar plot of line membership in each population was generated using structure 2.3.2.

For comparisons between the phenotypic and genotypic data, simple (r) and spearman rank (rs) correlation coefficient was calculated between the Euclidean distances for each variety, across all the datasets. The 'P' value for the correlation coefficient was calculated according to each distribution. To determine the similarity between the dataset, each pair was tested using the Mantel Z statistic [22]. The test was carried out using the R package ade4 [23], which calculate the Z value and the corresponding P value. Significance of Z was determined by comparing the observed Z values obtained and by calculating Z for one matrix with 2000 permuted variants of second matrix.

## 3. RESULTS AND DISCUSSION

### 3.1 Morphological Characterization

Characterization and genetic diversity assessment of elite rice varieties are important in view of identification and selecting suitable parental lines to develop new varietal combinations as well as, for protecting genotypes from unauthorized exploitation. Modern breeding makes the use of natural processes but takes full advantage of available genetic diversity to create new genotypes [24] and this needs clear-cut information on the extent of genetic diversity available so that the breeder can exploit the genotypes efficiently. Characterization of 24 elite Indian rice varieties in the present study showed considerable variation in all morpho-grain traits. The details of qualitative and quantitative morpho-grain traits for plant morphology and grain quality traits observed and their distribution among the varieties are shown in the Table 2 and 3. In case of quantitative traits, the coefficient of variation ranged from 8.01% (panicle length of main axis) to 25.67 per cent (stem thickness) among the varieties (Table 4). The highest variability was observed for stem thickness (25.67) followed by leaf length (21.05%) and grain weight (19.47%). The leaf length among the varieties ranged from 19.5 cm (RNR 23064) to 47.50 cm (WGL 13400). The lowest (0.85 cm) and the highest (1.45 cm) leaf width were observed in Tellahamsa and MTU

5249, respectively. Among the quantitative traits, time of heading which ranged from 78 days (RDR 7555) to 122 days (MTU 5249), the lowest stem length (60.50 cm) was observed in RDR 7555, while MTU 1010 showed the highest (99.0 cm). Kalajeera was characterized with the lowest stem thickness (0. 54 cm) against the highest in ASD 19 (1.15 cm). The varieties Tellahamsa and MTU 2077 respectively displayed the shortest (102.66 days) and the longest (147.33 days) time of maturity, respectively. Leaf and stem characteristics are important to distinguish the rice cultivars. The variation in the time of heading has been reported as a useful trait by Nethra [25]. According to national DUS testing guidelines of plant protection Act of India and also as per the present study, the stem length was less suitable for classification of the varieties. One of the most distinguishable features of cultivated rice was variation in the size and shape of grains. Among the 11 qualitative traits, only panicle awn trait displayed no variation among the varieties except WGL 20471. The rice varieties were grouped into five categories based on grain length, viz., very short (Kalajeera), short (ADT 43, BPT 5204, GEB 24, JGL 384, JGL 1798, Mahsuri, MTU 7029 and RDR 7555), medium (ASD 19, MTU 1001, MTU 2067, MTU 2077, MTU 5870, MTU 5249, RDR 763, WGL 14 and RNR 23064) and long (IR 64, MTU 1010, WGL 13400, WGL 20471 and RPW6-17) and very long (Tellahamsa). The grain traits showed considerable variation among all the varieties (Table 3). The seed weight was lowest in JGL 384 (13.5 g), while the variety RPW6-17 showed the highest (26.5 g). The variety Kalajeera with the lowest grain length (6.02 mm) whereas, Tellahamsa had shown having the highest (10.23 mm). Similarly, in BPT 5204, the grain width was very narrow (1.80 mm) and in RDR 7555it was broadest (3.05 mm). Based on the decorticated grain length, the varieties ranged from 4.53 (Kalajeera) to 6.90 mm (WGL 13400), while the decorticated grain width was very narrow (1.78 mm) in WGL 20471and very broad (2.70 mm) in RDR 7555. Panicle and grain characters such as the presence of awns, length and width of grain as well as for decorticated grain shape of grains were useful for variety characterization. In the present study, the length to width ratio ranged from 2.72 (land race Kalajeera) to 4.32 (Tellahamsa). According to Adair et al. [26], grain size and shape are important components of rice quality in developing new varieties and also for determining consumer acceptance. All the short and medium slender varieties were late in flowering and also showed considerable variations between these varieties and other varieties of rice. The amylose content in rice genotypes, influenced by amylose: Amylopectin ratio is a useful measure to characterize rice genotype [27]. In the current study, all the varieties possessed medium amylose content except MTU 1010, RDR 7555 and Tellahamsa which showed low and high amylose content respectively.

The broad morpho-grain trait diversity existed among the varieties in the present study indicated fair distribution of variation across all of the morphological traits. The significance of agro-morphological characters in diversity assessment of rice varieties has been well documented [28]. In agreement with Singhal and Prakash [29], a set of morphological traits are useful to distinguish the varieties from each other. A set of 12 morphological traits viz., altitude of flag leaf blade, time of heading and maturity, stem length and thickness, leaf traits and grain traits like grain weight, length and width of seed, shape of grain, shape of decorticated grain and gelatinization temperature were useful in characterization of the elite Indian rice varieties.

## 3.2 Principal Component Analysis (PCA)

The principal characters with higher Eigen vectors that delineated the varieties into separate groups in the first seven components are presented in Table 5. The 24 varieties were scattered by their component scores of the first seven principal components (PCs) with Eigen value higher than one and explained a total of 75.8% of their phenotypic variation components. The first PC explained 18.32% of the total variation, mainly related to quantitative variation of leaf length and width, time of heading, stem thickness, time of maturity and qualitative traits like intensity of green leaf colour, pubescence of leaf blade, attitude of culm and gelatinization temperature. In the second PC, which explained 14.59% of the total variation, predominant traits were leaf length and width of blade, time of heading, stem length, time of maturity, flag leaf attitude of leaf blade, panicle curvature of main axis, and grain traits (grain weight, grain and decorticated grain width). The third principal component, which accounted for 12.04% of the total variation, was dominated by traits such as leaf and stem traits, time of maturity, panicle number per plant, grain and decorticated grain length and shape, flag leaf attitude. While leaf width of blade, stem thickness, time of maturity,

grain length, leaf pubescence of blade surface and grain quality traits (grain weight, grain width and decorticated grain width, endosperm amylose content and gelatinization temperature) were important delineating traits associated with the fourth principal component, which documented 10.99% of the total variation. Principal components 5, 6 and 7 accounted for 8.32%, 6.23% and 5.39% respectively of the total variation and the main characteristics of these components were positively related to the width of leaf blade, stem length, panicle length of main axis, leaf pubescence of blade surface, panicle awn, stem thickness and flag leaf attitude of blade. The first three PCs, which contributed to 45% of the total variation, were plotted graphically to demonstrate the relationships between varieties (Fig. 1). The plot evidenced considerable diversity among the varieties. However, PCA could considerably differentiate the varieties according to their pedigree and grain types and the varieties did not form clear-cut distinct groups. The varieties considered for the present study have been developed to suit different rice growing ecosystems and consumer preferences based on their grain type. There were eight short and medium slender types and a landrace cultivars *viz*., ADT 43, BPT 5204, JGL 384, JGL 1798, Mahsuri, WGL 14, MTU 7029, RDR 763 and a landrace Kalajeera, which had higher factor loadings (>1) in PC1 compared to other medium and long grain varieties. These were distinct and formed the most divergent single line group compared to medium and long grain varieties for PC1 while, the medium and long slender and bold varieties released from Andhra Pradesh Rice Research station other than RPW 6-17 and WGL 13400 (MTU 1001, MTU 2067, MTU 2077, MTU 4870, MTU 5249, MTU 7029, RPW 6-17 and WGL 13400) are markedly distinct from the others in the PC2. The rest of the varieties *viz*., ASD 19, GEB 24, RDR 7555 and RNR 2306 showed higher factor loadings for PC3. The PC1 through PC7 vectors explains progressively less of the variation across the plant morphology and grain quality characteristics.

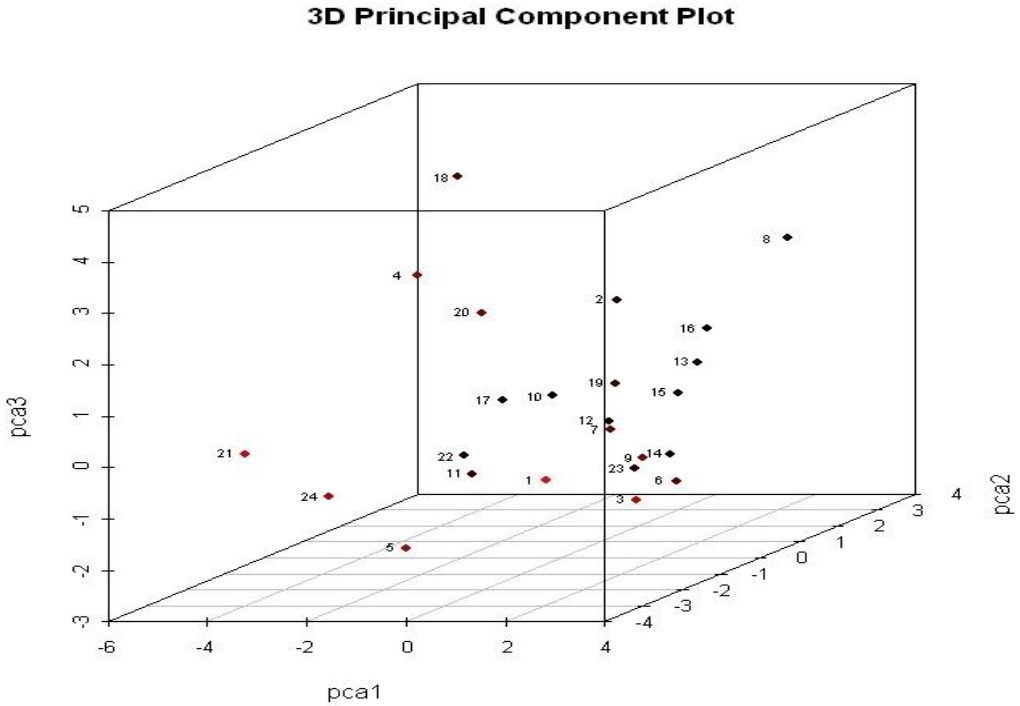


**Fig. 1. Scatter plot of the three principal components (PC1, PC2 and PC3) using agro morphological traits (1-ADT 43; 2-ASD 19; 3-BPT 5204; 4-GEB 24; 5-IR 64; 6-JGL 384; 7-JGL 1798; 8-Kalajeera; 9-Mahsuri; 10-MTU 1001; 11-MTU 1010; 12-MTU 2067; 13-MTU 2077; 14-MTU 4870; 15-MTU 5249; 16-MTU 7029; 17-RPW6-17; 18-RDR 7555; 19-RDR 763; 20-RNR 23064; 21-Tellahamsa; 22-WGL 13400; 23-WGL 14; 24-WGL 20471**

**Table 2. Plant morphological traits among elite Indian rice varieties**

| Variety | Leaf intensity of green colour | Leaf pubescence of blade surface | Attitude of culm | Flag leaf attitude of blade (early observation) | Flag leaf attitude of blade (late observation) | Panicle curvature of main axis | Panicle awn | Panicle exsertion | Leaf length of blade | Leaf width of blade | Time of heading | Stem length | Stem thickness | Panicle length of main axis | Panicle number per plant | Time of maturity (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ADT 43 | Light | Weak | Semi-erect | Erect | Erect | Semi-straight | Absent | Exserted | Long | Medium | Early | Very short | Thick | Medium | Medium | Early |
| ASD 19 | Dark | Medium | Semi-erect | Horizontal | Deflexed | Drooping | Absent | Well exserted | Long | Medium | Medium | Very short | Thick | Short | Medium | Medium |
| BPT 5204 | Medium | Medium | Open | Erect | Semi-erect | Semi-straight | Absent | Exserted | Long | Narrow | Late | Very short | Thick | Short | Medium | Late |
| JGL 384 | Medium | Medium | Semi-erect | Semi-erect | Semi-erect | Semi-straight | Absent | Exserted | Long | Medium | Late | Very short | Thick | Long | Medium | Late |
| JGL 1798 | Medium | Medium | Semi-erect | Erect | Horizontal | Semi-straight | Absent | Well exserted | Medium | Medium | Medium | Very short | Thick | Long | Medium | Medium |
| GEB 24 | Light | Medium | Open | Horizontal | Deflexed | Deflexed | Absent | Well exserted | Long | Narrow | Early | Very short | Medium | Long | Medium | Early |
| Mahsuri | Dark | Medium | Semi-erect | Erect | Semi-erect | Semi-straight | Absent | Exserted | Long | Narrow | Medium | Very short | Thick | Long | Medium | Medium |
| IR 64 | Medium | Strong | Semi-erect | Erect | Semi-erect | Drooping | Absent | Exserted | Long | Medium | Medium | Very short | Thick | Long | Medium | Medium |
| MTU 1010 | Medium | Medium | Erect | Semi-erect | Deflexed | Drooping | Absent | Well exserted | Long | Narrow | Medium | Short | Thick | Long | Medium | Medium |
| MTU 2077 | Medium | Very strong | Erect | Semi-erect | Horizontal | Drooping | Absent | Exserted | Long | Medium | Late | Very short | Thick | Long | Medium | Late |
| MTU 5249 | Dark | Strong | Erect | Semi-erect | Horizontal | Drooping | Absent | Exserted | Long | Medium | Late | Short | Thick | Long | Medium | Late |
| MTU 4870 | Medium | Medium | Semi-erect | Erect | Semi-erect | Semi-straight | Absent | Exserted | Long | Medium | Late | Short | Thick | Medium | Medium | Late |
| MTU 1001 | Medium | Weak | Semi-erect | Erect | Horizontal | Drooping | Absent | Well exserted | Long | Medium | Late | Short | Thick | Long | Medium | Late |
| MTU 2067 | Medium | Medium | Semi-erect | Semi-erect | Horizontal | Semi-straight | Absent | Exserted | Long | Medium | Late | Very short | Thick | Long | Medium | Late |
| MTU 7029 | Dark | Strong | Semi-erect | Semi-erect | Horizontal | Drooping | Absent | Partly exserted | Medium | Medium | Late | Very short | Thick | Long | Medium | Late |
| WGL 14 | Medium | Medium | Semi-erect | Semi-erect | Semi-erect | Drooping | Absent | Exserted | Long | Medium | Late | Very short | Thick | Long | Medium | Late |
| RPW 6-17 | Dark | Medium | Semi-erect | Semi-erect | Horizontal | Drooping | Absent | Exserted | Long | Narrow | Late | Very short | Thick | Long | Medium | Late |
| WGL 13400 | Medium | Medium | Erect | Semi-erect | Semi-erect | Drooping | Absent | Well exserted | Long | Medium | Medium | Very short | Medium | Long | Medium | Late |

| Variety | Leaf intensity of green colour | Leaf pubescence of blade surface | Attitude of culm | Flag leaf attitude of blade (early observation) | Flag leaf attitude of blade (late observation) | Panicle curvature of main axis | Panicle awn | Panicle exsertion | Leaf length of blade | Leaf width of blade | Time of heading | Stem length | Stem thickness | Panicle length of main axis | Panicle number per plant | Time of maturity (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WGL 20471 | Medium | Medium | Semi-erect | Semi-erect | Horizontal | Drooping | Present | Exserted | Long | Medium | Early | Very short | Thick | Long | Medium | Early |
| RDR 7555 | Medium | Medium | Semi-erect | Semi-erect | Horizontal | Drooping | Absent | Exserted | Medium | Medium | Early | Very short | Thick | Long | Medium | Very early |
| RDR 763 | Medium | Very strong | Erect | Erect | Horizontal | Drooping | Absent | Well exserted | Long | Medium | Medium | Very short | Medium | Long | Medium | Medium |
| Tellahamsa | Medium | Absent | Semi-erect | Semi-erect | Horizontal | Semi-straight | Absent | Well exserted | Long | Narrow | Early | Short | Medium | Long | Medium | Very early |
| RNR 23064 | Medium | Medium | Semi-erect | Semi-erect | Horizontal | Drooping | Absent | Well exserted | Short | Medium | Medium | Very short | Medium | Long | Medium | Medium |
| Kalajeera | Dark | Weak | Open | Semi-erect | Horizontal | Deflexed | Absent | Well exserted | Long | Narrow | Late | Very short | Medium | Long | Few | Late |

**Table 3. Grain morphological traits among elite Indian rice varieties**

| Variety | 1000 grain weight (g) | Grain length (mm) | Grain width (mm) | Decorticated grain length (mm) | Decorticated grain width (mm) | Decorticated grain shape | Endosperm amylose content | Gelatinization of temperature |
|---|---|---|---|---|---|---|---|---|
| ADT 43 | Very low | Small | Narrow | Small | Narrow | Medium slender | Medium | High intermediate |
| ASD 19 | Low | Medium | Medium | Small | Narrow | Short slender | Medium | Intermediate |
| BPT 5204 | Low | Small | Narrow | Small | Narrow | Medium slender | Medium | High intermediate |
| JGL 384 | Very low | Small | Narrow | Small | Narrow | Medium slender | Medium | Intermediate |
| JGL 1798 | Low | Small | Narrow | Small | Narrow | Medium slender | Medium | Intermediate |
| GEB 24 | Low | Small | Narrow | Small | Medium | Medium slender | Medium | Intermediate |
| Mahsuri | Low | Small | Medium | Small | Medium | Medium slender | Medium | High intermediate |
| IR 64 | Medium | Long | Medium | Medium | Medium | Long bold | Medium | High intermediate |
| MTU 1010 | Medium | Long | Narrow | Medium | Medium | Long bold | Low | High intermediate |
| MTU 2077 | Low | Medium | Medium | Small | Medium | Medium slender | Medium | Intermediate |
| MTU 5249 | Low | Medium | Medium | Medium | Medium | Long bold | Medium | Intermediate |
| MTU 4870 | Medium | Medium | Medium | Small | Medium | Medium slender | Medium | Low |
| MTU 1001 | Medium | Medium | Medium | Medium | Medium | Medium slender | Medium | Low |
| MTU 2067 | Low | Medium | Medium | Small | Medium | Medium slender | Medium | Intermediate |
| MTU 7029 | Low | Small | Narrow | Small | Medium | Shot bold | Medium | Intermediate |
| WGL 14 | Very low | Medium | Medium | Small | Narrow | Medium slender | Medium | Intermediate |
| RPW 6-17 | High | Long | Broad | Medium | Medium | Long bold | Medium | Low |
| WGL 13400 | High | Long | Medium | Medium | Medium | Long slender | Medium | Intermediate |
| WGL 20471 | Medium | Long | Very narrow | Medium | Narrow | Long bold | Medium | Low |

| Variety | 1000 grain weight (g) | Grain length (mm) | Grain width (mm) | Decorticated grain length (mm) | Decorticated grain width (mm) | Decorticated grain shape | Endosperm amylose content | Gelatinization of temperature |
|---|---|---|---|---|---|---|---|---|
| RDR 7555 | Medium | Small | Very broad | Small | Medium | Short bold | Low | Intermediate |
| RDR 763 | Low | Medium | Narrow | Small | Narrow | Short slender | Medium | Intermediate |
| Tellahamsa | Medium | Very long | Medium | Medium | Medium | Long slender | High | Low |
| RNR 23064 | Low | Medium | Narrow | Small | Medium | Medium slender | Medium | Intermediate |
| Kalajeera | Very low | Very small | Narrow | Small | Medium | Short slender | Medium | High intermediate |

**Table 4. Descriptive statistics for 13 morpho-grain quantitative traits among elite Indian rice varieties**

| Trait | Mean±SD | Range | C.V |
|---|---|---|---|
| Leaf length of blade (cm) | 36.97±7.9 | 19.5 – 47.50 | 21.05 |
| Leaf width of blade (cm) | 1.16±0.2 | 0.85 – 1.45 | 17.31 |
| Time of heading (days) | 104.04±15.2 | 77.66 – 122.33 | 12.64 |
| Stem thickness (cm) | 0.80±0.2 | 0. 54 – 1.15 | 25.67 |
| Stem length (cm) | 78.91±12.0 | 60.5 – 99.0 | 15.22 |
| Panicle length of main axis (cm) | 22.12±1.8 | 19.62 – 25.49 | 8.01 |
| Panicle number per plant | 16.79±2.1 | 14.00 – 19.00 | 12.68 |
| Time of maturity (days) | 131.56±13.2 | 102.66 – 147.33 | 12.20 |
| 1000 Grain weight (g) | 18.99±3.7 | 13.5 – 26.5 | 19.47 |
| Grain length (mm) | 8.27±0.9 | 6.02 – 10.23 | 11.46 |
| Grain width (mm) | 2.29±0.2 | 1.80– 3.05 | 10.89 |
| Decorticated grain length (mm) | 5.89±0.6 | 4.53 - 6.90 | 9.84 |
| Decorticated grain width (mm) | 2.09±0.2 | 1.78 – 2.7 | 10.22 |

The combined PCA of the qualitative and quantitative morpho-grain traits grouped most of the varieties into one group, while a few others remained scattered. However, the Eigen values spread up to seven vectors, showed a total of 75.8% of their phenotypic variation components. In concurrence with Meesang et al. [3] this may be due to the use of highly adapted genotypes with less phenotypical distinction for breeding programmes. In PCA of the present study, the first three PCs predominantly explained the total variance that was observed to a considerable degree of distribution based on plant morpho-grain traits. The landrace Kalajeera stood apart from all other varieties in the PCA plot which may be due to its distinct origin, Eastern or North Eastern India (www.mssrf.org/bd/ebooks/ebook8.pdf) and high genetic similarity to *japonica* than *indica*. Further, Oka and Chang [30] also noticed wide variations between landraces and cultivated types in the rice populations of tropical regions. The varieties, RDR 7555, GEB 24, ASD 19, RNR 23064, Tellahamsa, WGL 20471, IR 64 and Kalajeera were distinct from the others and remained scattered across the plot reflecting the important morphological differences among them accounted by leaf, time of heading and maturity, panicle, decorticated grain length and gelatinization temperature. Though the cluster analysis in general was consistent with that of PCA, many of the short and medium slender varieties displayed a tendency to stay together. In conformity with Fuentes et al. [31], the diversity among varieties can be estimated efficiently based on the parentage and phenotypic diversity, permitting a more effective separation of the progenitor set than those obtained solely by phenotypic or genealogical information.

The average intra and inter-cluster distance values for varieties based on Euclidean distances are presented Fig. 2. Intra-cluster Euclidean values ranged from 55.1 (cluster II) to 0.0 (cluster V). Based on the inter-cluster Euclidean values, the highest divergence (100.01) occurred between cluster II and V, while the lowest divergence (47.81) was between cluster III and IV. The inter-cluster distance between I and II and III and IV, were moderately divergent. Cluster analysis based on Euclidean distances of morphological traits classified the varieties in the present study into five major groups and it revealed considerable diversity among the rice cultivars. The varieties JGL 384, MTU 2077, BPT 5204 and MTU 4870 in group 1; varieties RNR 23064 and Kalajeera in group 2; MTU 7029, WGL 13400, JGL 1798, Tellahamsa, ADT 43, MTU 1010, RPW 6-17, MTU 5249, GEB 24 and MTU 1001 in group 3 and the varieties RDR 763, IR 64, Mahsuri, MTU 2067, WGL 20471, ASD 19 and WGL 14 showed the closest morphological similarity and were placed in group 4 while, RDR 7555 variety was in group 5 and was separated from other varieties. The group 2 and 5 were morphologically different with respect to many of the traits characterized. The highest inter-cluster distance observed between II and V clusters (100.01) was due to the variety RNR 23064 and the landrace Kalajeera, while the lowest inter-cluster distance (47.81) between III and IV clusters may be due to the close genetic relationship among the varieties, similar parentages or grain type. The specific groupings of the varieties according to the pedigree and grain type up to some extent indicated the importance of these morphological traits for genetic diversity analysis. For eg., the cultivars BPT 5204 (GEB 24/TN1/Mahsuri) and JGL 384 (Kavya/BPT 5204) and MTU 1001 (MTU 5249 x MTU 7014) and MTU 5249 (MTU 4569 x ARC 6650) showed grouping according to their pedigree and shared at least one common parent in their pedigree.

### 3.3 Molecular Characterization

A total of 251 alleles were amplified using 86 SSR markers distributed across the genome (primers/chromosome) of 24 rice varieties. The number of alleles amplified by each primer pair ranged from 2 to 6 with an average of 3.0. The polymorphism information content (PIC) for these markers ranged from 0.54 (RM 13) to 0.97 (RM 226). The markers *viz*., RM 226, RM 11229, RM 16153 and RM 15630 amplified the highest number of alleles (6 each), and was followed by 5 each in RM 204, RM 307, RM 16649, and RM 333. The SSR markers RM 14140, RM 16913, RM 6759, RM 70, RM 324, RM 336, RM 248, RM 72, RM 206, RM 247 and RM 20 amplified 4 alleles each. The number of alleles observed for 24 elite varieties of rice was analyzed in the study. The Jaccard's similarity coefficient ranged from 0.26 (JGL 1798 Vs JGL 384) to 0.76 (Kalajeera Vs RDR 7555) with an average similarity index of 0.52. In pair-wise comparisons, least similarity index was observed between the JGL 384 and JGL 1798 (0.26) whereas, landrace Kalajeera and RDR 7555 showed least similarity index (0.76). The Fig. 3 shows the dendrogram of 24 rice varieties based on SSR amplification pattern, the varieties were observed to be

grouped into five major clusters. Major cluster I consisting of JGL 384, JGL 1798, BPT 5204, WGL 14, RDR 763, RNR 23064, ADT 43, ASD 19, GEB 24, Mahsuri, MTU 7029, MTU 7029 and IR 64 had 73.30% similarity, whereas cluster II with varieties Phalguna (RPW6-17), WGL 13400, Tellahamsa and RDR 7555 had average similarity of 66.8% among them. Cluster III consisted of MTU 2077, MTU 2067, MTU 4870, MTU 1001 and MTU 5249 of long grain varieties with a similarity of 42.6%. The variety Erramallelu (WGL 20471) and a landrace, Kalajeera were distinct from each other and also from other cultivars and were separated into cluster IV and cluster V, respectively. In the present study, SSR markers grouped the varieties in each cluster predominantly according to their genetic background. Based on marker data, distinct clusters were formed with related varieties. The varieties JGL 384 and JGL 1798 were grouped together as they were developed using same parents for medium slender grain type. Similarly, medium slender grain type varieties, namely BPT 5204, WGL 14, RNR 23064 and a short slender grain type variety RDR 763 were grouped in the same cluster. But, these cultivars were not grouped together when morphological characteristics were employed. The poly-allelic nature of SSR markers is highly favourable to discriminate the individuals more precisely. SSR markers RM 226, RM 11229, RM 16153 and RM 15630 showed six alleles across the varieties which formed five major groups of different compositions. The varieties' having short, medium or long slender grain types released for commercial cultivation along with other cultivars in diverse geographical locations across India are expected to be genetically diverse, and it become obvious by looking at the range of similarity coefficient between the varieties (0.26 to 0.76). High degree of similarity (76%) observed between JGL 1798 Vs JGL 384 followed by WGL 13400 with RPW6-17 (75%) and BPT 5204 and WGL 14 and Tellahamsa and RDR 7555 (73%), mainly because of their closely related parental lines. Least similarity was observed between Kalajeera and the rest of varieties, as emphasized earlier based on the PCA and may be due to the distinct origin of Kalajeera (Eastern or North-Eastern India). The grouping of MTU 2077, MTU 2067, MTU 4870, MTU 1001, MTU 1010 and MTU 5249 into a cluster with similarity ranging from 0.34 to 0.71 is due to their close pedigree. Further, they were released from Regional Agricultural Research Station, Maruteru, Andhra Pradesh State, India using similarly adapted genotypes as parent material and the possibility of few varieties with one of the genotypes as a common parent. The short slender variety Mahsuri and short bold variety MTU 7029 were clustered in a group as the Mahsuri is the one of the parente for MTU 7029. Similarly, Tellahamsa and RDR 7555 were grouped in one cluster showed similarity as one of the parental lines was common among them and interestingly both of them possessed long and bold grain types. However, the varieties RDR 763 and BPT 5204 did not group together though they have one common parent. Also the varieties, IR 64, MTU 2077 and MTU 1010 with closely related parentage were not a part of the same cluster, which might be due to differential selection pressure and selection criteria adopted for the development of these varieties. Ravi et al. [32] demonstrated the efficacy of SSR markers in an accurate determination of relationship between closely related genotypes and to analyze varieties with close pedigrees. In the present study, out of 86 SSR markers analyzed, 14 most informative primers (RM 70, RM 204, RM 11229, RM 16153, RM 15630, RM 206, RM 217, RM 226, RM 16649, RM 248, RM 307, RM 324, RM 333 and RM 336) were identified for the fingerprinting of closely related elite rice varieties.

### 3.4 Population Structure

A model based statistical analysis STRUCTURE was performed using the data of 251 polymorphic loci to recognize the genetic structure and to determine if an alternative structure could be elucidated with molecular marker data among the 24 elite varieties as explained in the materials and methods. The study provided significant evidence for existence of a population structure in these rice varieties. In most of the cases, consistent estimates of P(X/K) were obtained across the independent runs. The optimum population structure was inferred based on $\Delta K$, an *ad hoc* parameter and three clusters were identified among the 24 varieties. The lines have been listed by their primary membership in each of these populations, where admixture values, Q, for the population of primary membership were <0.50, line was considered as mixed within their population of primary membership. Each line's estimated membership in each of the three populations is depicted in a STRUCTURE bar plot (Fig. 4) and exact membership proportions are provided in Table 6. Cluster 1 composed of 10 fine-grain (short and medium types) varieties except GEB 24, Mahsuri and Kalajeera; cluster 2 contained six coarse grain (long bold types) varieties except MTU

2077 and MTU 4870 medium slender varieties; while the cluster 3 comprised eight fine grain (short and medium slender) varieties. Population structure based on Q values with k=3 predominantly matched very well with UPGMA tree from the distance based analysis (Fig. 3). Differentiation estimated by F-statistics values between clusters determined by the software STRUCTURE is 0.0813, 0.0805 and 0.2079 in between cluster 1 and 2; 2 and 3 and 1 and 3 respectively, indicating to high differentiation. Genetic structure of populations has been previously documented in rice [33,34]. Based on the cluster analysis, Nelson et al. [34] identified six primary genetic clusters represented by genotypes. The present study, exhibited a structure with three clusters. Overall, the varieties were assigned into corresponding clusters with the membership proportion (Q) more than 0.53 except ADT 43 (0.47) in cluster 3. This variety also shared a secondary membership with cluster 1 (0.38). However, the short slender varieties GEB 24, MTU 7029, Mahsuri and landrace Kalajeera were grouped in cluster 1 and shared secondary membership with cluster 3 group (short and medium grain types). In some of these cases, the shared ancestry could be because of shared pedigree relationships and a perusal of the pedigree of these varieties indicates close relationship among the varieties. The simulations in *structure* population indicated that these varieties in the sub-population 1 could have received gametes from sub-population 3 as these are predominantly short and medium slender types.

### 3.5 Comparison of Phenotypic and Genotypic Markers Based on Diversity Estimates

The correlation between genotypic and phenotypic similarity value was moderately significant ($r = 0.36$ $P<10^{-4}$ ; $rs = 0.12$ $P<10^{-2}$). The Z-test statistic was also significant between GS and MS matrices (Z=0.39; P=0.0002). The results indicate a positive correspondence between the two similarity measures (Fig. 5) with the clusters of 24 cultivars of rice which were observed to be nearly similar in both the analyses. However, some discrepancies between the morphological traits (Fig. 2) and molecular dendrogram (Fig. 3) were observed, especially with respect to the varieties GEB 24, Tellahamsa, RPW 6-17, ASD 19, IR 64, MTU 7029 and RDR 7555. The three clusters based on genetic structure Q values and five clusters based on UPGMA with molecular markers for the 24 varieties corresponded largely to each other with respect to their pedigree relationship and morphological traits. However, contrary to the UPGMA clustering, the variety WGL 20471 and landrace Kalajeera were divided in subgroup 1 showing unknown linkage with *structure* population in this study.

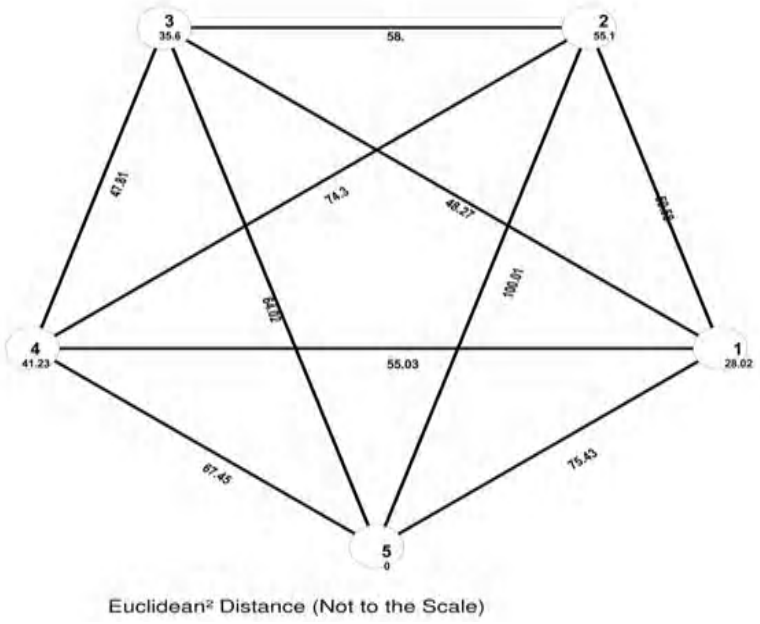


**Fig. 2. Euclidean distances among elite Indian rice varieties**

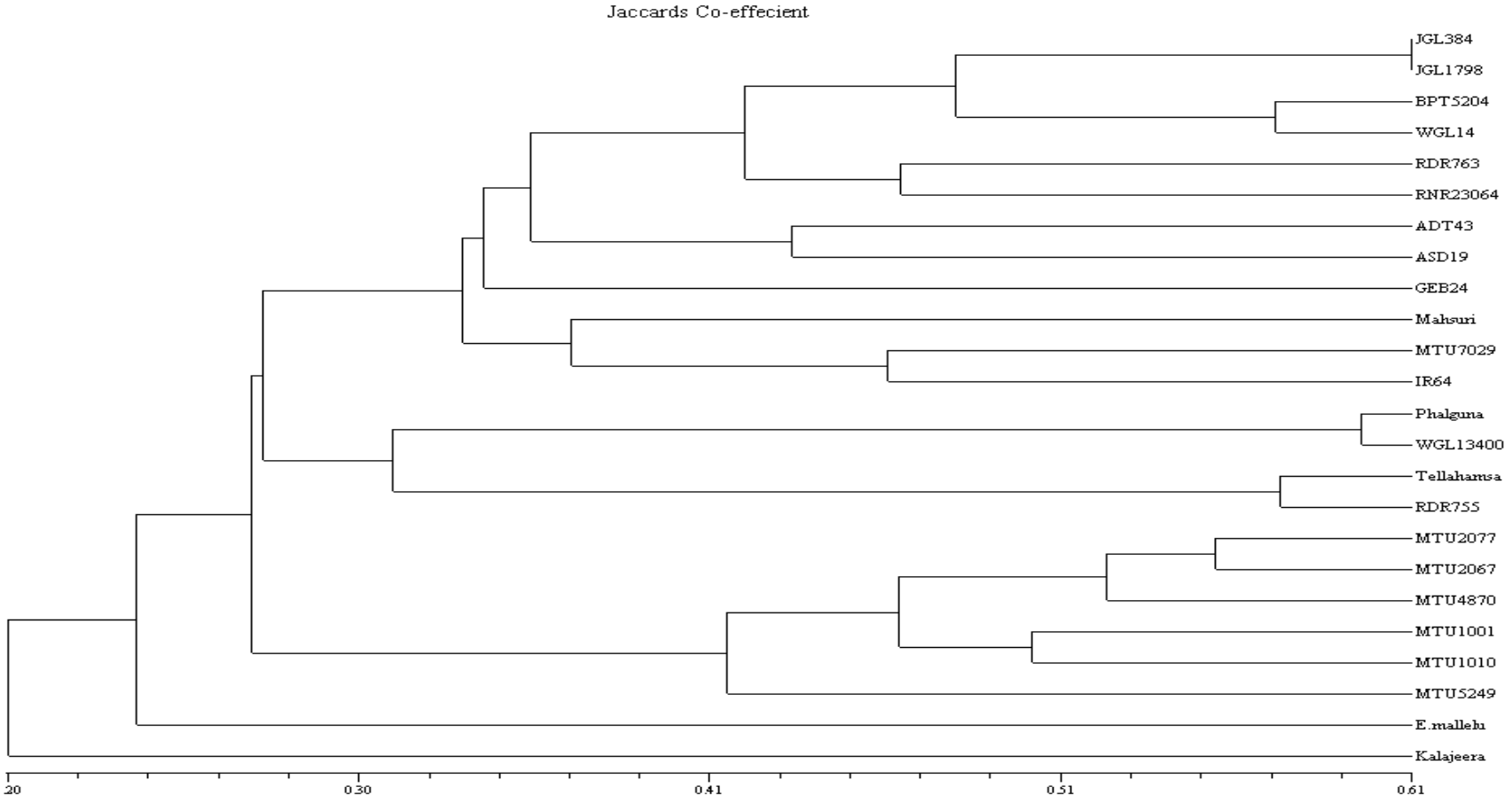


**Fig. 3. Dendrogram based on 256 SSR alleles derived from UPGMA cluster analysis using Jaccard's coefficient**

**Table 5. Contribution percentage and agro morphological traits associated with the seven principal components of elite Indian rice varieties and their Eigenvectors**

| Factor | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Contribution percentage (%) | 18.32 | 14.59 | 12.04 | 10.99 | 8.32 | 6.23 | 5.39 |
| **Characters** | **Eigenvector** | | | | | | |
| Length of leaf blade (cm) | 1.738 | 1.342 | 1.342 | -1.815 | -2.161 | -1.023 | 0.518 |
| Width of Leaf blade (cm) | 1.059 | 1.527 | 1.400 | 1.991 | 2.345 | -1.719 | -0.661 |
| Time of heading (days) | 3.739 | 1.785 | 1.562 | -0.927 | -0.608 | -0.417 | 0.093 |
| Stem length (cm) | -0.406 | 0.909 | 1.289 | -1.686 | -1.772 | 1.136 | -0.253 |
| Stem thickness (cm) | 1.959 | -2.893 | 1.210 | 1.840 | 0.263 | 2.050 | -1.238 |
| Panicle length of main axis (cm) | -0.946 | -1.347 | 0.134 | -1.240 | 1.076 | -1.495 | 1.616 |
| Panicle number per plant | -0.233 | -3.140 | 1.845 | 0.523 | -1.226 | 0.212 | 0.135 |
| Time of maturity (days) | 4.043 | 1.351 | 1.332 | -0.985 | -0.226 | -0.937 | 0.108 |
| 1000 grain weight(g) | -2.703 | 1.324 | 0.798 | 1.268 | -0.251 | 0.386 | 0.772 |
| Grain length (mm) | -3.401 | 0.197 | 1.968 | -0.538 | 0.344 | 0.653 | -0.504 |
| Grain width (mm) | -1.135 | 2.521 | -1.137 | 2.545 | -0.781 | 0.602 | 0.434 |
| Decorticated grain length (mm) | -3.135 | 0.342 | 2.312 | -0.541 | 0.583 | 0.654 | -0.084 |
| Decorticated grain width (mm) | -0.199 | 2.720 | -1.214 | 2.603 | -0.916 | 0.163 | 0.962 |
| Intensity of green leaf Colour | 2.266 | 1.303 | -0.197 | 0.760 | -0.378 | 1.242 | -1.274 |
| Pubescence of leaf blade surface | 2.001 | 0.406 | 0.253 | 0.298 | 3.636 | 0.526 | 0.441 |
| Attitude of culm | 0.760 | -3.345 | -1.647 | 0.325 | -2.050 | -2.137 | 0.363 |
| Flag leaf attitude of blade early observation | -0.442 | 0.590 | -2.633 | -1.414 | 0.345 | 0.034 | 0.474 |
| Flag leaf attitude of blade late observation | -0.858 | 1.320 | -2.579 | -1.781 | 0.460 | 1.557 | -0.796 |
| Curvature of main panicle axis | 0.082 | 0.921 | -2.866 | -1.832 | 0.818 | -0.277 | 1.069 |
| Panicle awn | -1.170 | -1.381 | -0.742 | -0.549 | 2.145 | -1.972 | -2.227 |
| Panicle exsertion | -1.014 | -0.441 | -1.561 | -2.109 | -1.113 | 0.335 | -2.540 |
| Decorticated grain shape | -2.690 | -0.390 | 2.487 | -0.801 | -0.059 | -0.683 | 0.691 |
| Endosperm amylose content | -1.214 | -0.283 | -1.519 | 3.426 | -1.303 | -1.536 | -1.016 |
| Gelatinization of temperature | 2.652 | -3.143 | -0.793 | 0.471 | 0.321 | 1.617 | 0.732 |

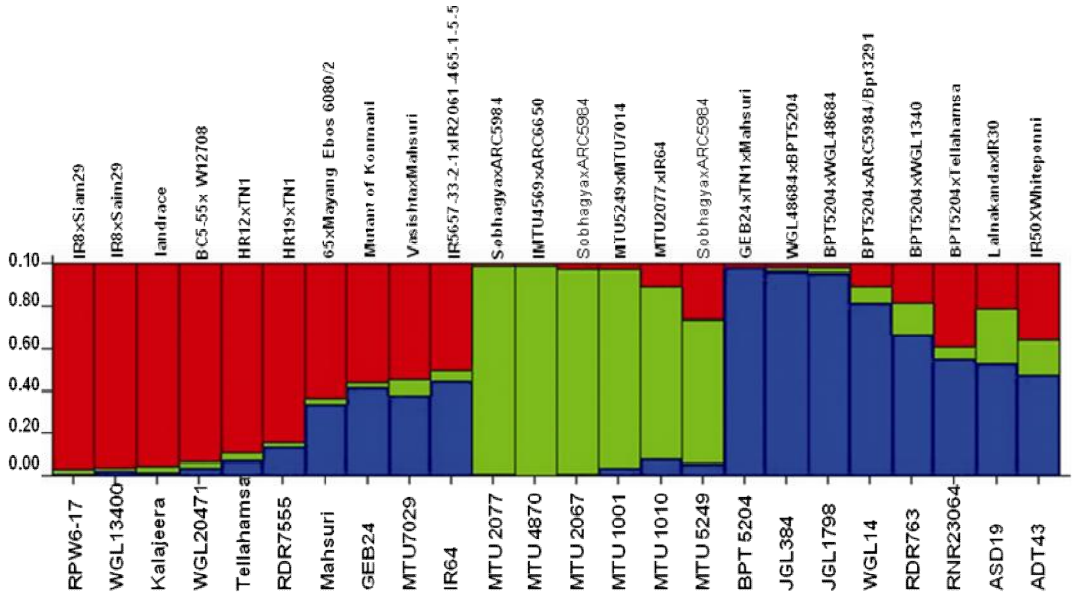


**Fig. 4. Estimation of population structure based on 86 SSR markers; each variety in a population is represented by bar plot proportionally to Q values of 24 elite Indian varieties up to three colours into three subpopulation based on their pedigree**

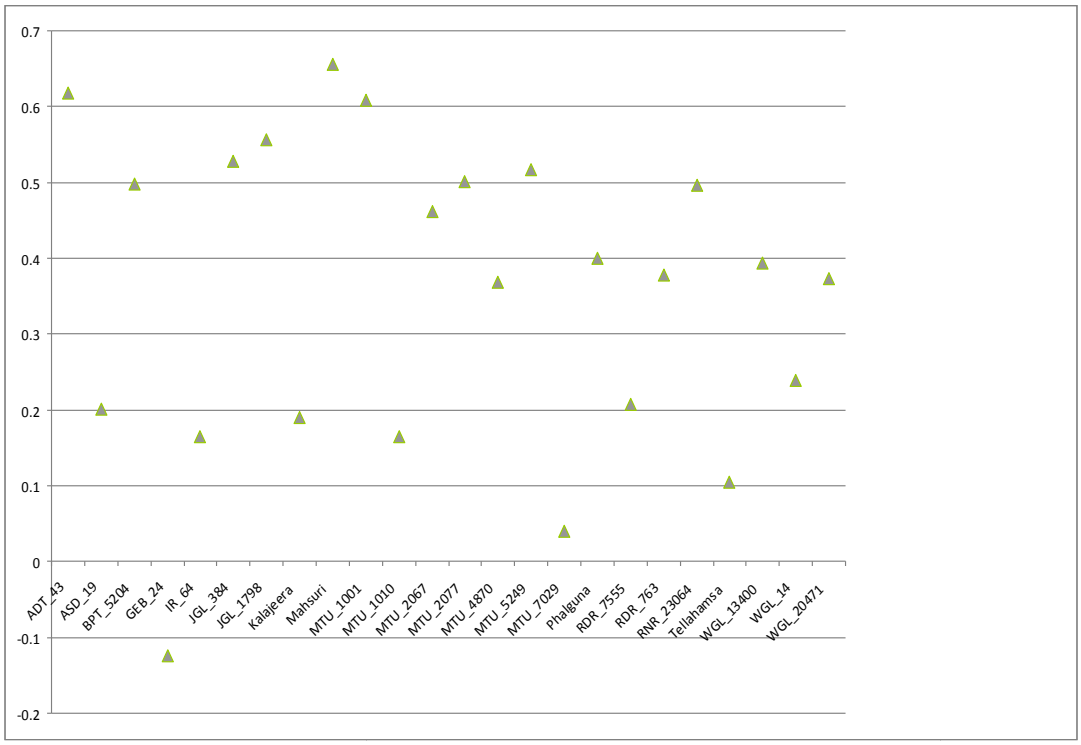


**Fig. 5. Correlation between of SSR marker and morphological traits among elite Indian rice varieties**

**Table 6. Estimated membership inference cluster values among rice varieties**

| Variety | Cluster 1 | Cluster 2 | Cluster 3 |
|---|---|---|---|
| JGL 384 | 0.016 | 0.013 | 0.971 |
| JGL 1798 | 0.017 | 0.024 | 0.959 |
| BPT 5204 | 0.014 | 0.005 | 0.981 |
| WGL 14 | 0.100 | 0.078 | 0.822 |
| RDR 763 | 0.190 | 0.146 | 0.664 |
| RNR 2306 | 0.402 | 0.051 | 0.548 |
| MTU 2077 | 0.010 | 0.981 | 0.009 |
| MTU 4870 | 0.013 | 0.98 | 0.007 |
| MTU 1001 | 0.027 | 0.934 | 0.039 |
| MTU 2067 | 0.026 | 0.963 | 0.011 |
| MTU 7029 | 0.558 | 0.077 | 0.366 |
| MTU 1010 | 0.123 | 0.811 | 0.066 |
| MTU 5249 | 0.277 | 0.667 | 0.055 |
| RPW6-17 | 0.971 | 0.02 | 0.009 |
| WGL 134 | 0.965 | 0.008 | 0.027 |
| WGL 20471 | 0.946 | 0.021 | 0.033 |
| Tellahamsa | 0.894 | 0.034 | 0.071 |
| RDR 7555 | 0.838 | 0.018 | 0.144 |
| ADT 43 | 0.385 | 0.141 | 0.474 |
| ASD 19 | 0.253 | 0.235 | 0.512 |
| GEB24 | 0.576 | 0.023 | 0.401 |
| Mahsuri | 0.655 | 0.024 | 0.321 |
| IR 64 | 0.526 | 0.044 | 0.430 |
| Kalajeera | 0.961 | 0.027 | 0.012 |

The correlation between morphological and SSR marker data exhibited similar grouping among the varieties. Three consistent groups were found in both the diversity classifications. The first group consisted, the bold and long slender grain cultivars including a landrace Kalajeera except WGL 14 and ASD 19, the second group consisted of all the medium and long slender MTU varieties developed by the Andhra Pradesh State Rice Research Institute of India. The third group was composed by the short slender varieties *viz*., JGL 384, JGL 1798, BPT 5204, ADT 43 and Mahsuri. The other varieties were so diverse with respect to their genetic background and therefore, no consistent relationship could be found between the data derived from SSRs and morphological traits. Similarly, high correspondence between phenotypic and genotypic diversities has been documented among rice varieties [31]. The significant correlation observed in the present study between the two diversity measures could be possibly explained by the SSR markers that are being associated with chromosomal regions which could have selected for particular environments and morphological traits relevant to adaptation.

## 4. CONCLUSION

SSR markers provide a greater allelic discrimination for the characterization of elite Indian rice varieties as compared to morpho-grain traits. Agro-morphological traits can be reliably deployed along with SSR markers for the characterization and genetic diversity assessment of rice varieties and can also be used to design better experiments in DUS testing to assess distinction.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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