Genetic Diversity of Korean Rice (Oryza Sativa L.) Germplasm for Yield and Yield Related Traits for Adoption in Rice Farming System in Nigeria

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Abstract: Background and objectives Assessment of genetic diversity is a prerequisite for any crop improvement program. It helps plant breeders in identifying promising lines for possible crosses. Materials and methods: This study was carried out at AfricaRice Center, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria, and evaluated 123 accessions from South Korea with 7 genotypes form Africa. The experiment was conducted in dry season using Alpha lattice design with 26 blocks each planted in five entries, replicated two times. Results: PCA showed that the first four components accounted for 73.59% of the total variation. Thus, suggest the presence of large genetic variability, which is of important, as it gives wide spectrum of selection to the breeders. Among all genotypes UPN296, UPN248 and UPN272 showed higher number of productive tillers, while UPN255, UPN332, and UPN 285 were superior for 1000-grain weight. The genotypes such as UPN277 and UPN261 proved to be better for number of spikelets, while UPN347, UPN266, and UPIA2 were better for grain yield. Cluster analysis grouped the 130 genotypes into 4 clusters. All the 17 SSRs markers used were polymorphic. A total of 70 alleles were obtained with an average of 4.12, and ranged from 2 to 6. PIC values ranged from 0.34 to 0.76 with an average of 0.53 with 17 SSR markers. UPGMA dendrogram based on similarity index of simple matching grouped 130 genotypes into three clusters. Conclusion. UPN347, UPN277, UPN296, UPN255 and UPIA2 shown to be the most promising genotypes that could be used for rice hybridization, genetic improvement and rice hybrid programme in Nigeria.

Keywords: Rice Genotype, Genetic Diversity, PIC, SSR Marker

1. Introduction

Rice (Oryza sativa) is the most widely grown and the staple food of an estimated 3.5 billion people worldwide [1] however food insecurity remains a serious global problem. The world population is predicted to reach nine billion by the year 2050 [2], but already by the year 2035, an estimate of 116 Mt of milled rice will be needed worldwide [3] to meet the increasing in demand. In Africa, where rice is the most rapidly growing food, about 30 million tons of rice will be required to feed the population, representing an increase of 130% in rice consumption from 2010 [4]. In Africa, rice is cultivated in 40 countries on nearly 10 million hectares in rain-fed upland and aquatic ecologies [5]. Although, West Africa has about 4.4 million hectares planted to rice [6], there is a production deficit due to high demand, which surpasses local production. To meet the food demand of the growing population and to achieve food security, the present production level needs to be increased. For a successful breeding program, germplasm evaluation is a pre-requisite on which the future actions could be taken in any crop

improvement programme.

Molecular markers are one of the tools for genetic resources characterization and grouping into distinct cluster based on genetic distance, thereby increasing the efficiency of breeding [7]. There should be a good understanding of the relationship between genotypic and phenotypic variation, which is key in the application of genomics in plant improvement [8]. Recent techniques, such as DNA-based markers, provide highly effective and reliable tools for evaluating genetic diversity in rice crop germplasm. Simple Sequence Repeat (SSR) is one of the most DNA molecular markers widely used to evaluate the genetic diversity and establishment of unique fingerprint owing to their abundance, codominant inheritance, high polymorphism, reproducibility, ease of assay by (PCR) polymerase chain reaction [9]. SSR markers have been effectively utilized for many purposes in rice production: genetic diversity and relatedness [10], QTL mapping [11], marker assisted selection and rice domestication [12]. With this background, the study was undertaken to assess genetic diversity among Korea lines and Africa adapted rice varieties using morphological traits and molecular markers and identify the promising lines and also, based on cluster analysis for their introgression into the existing rice breeding programme.

2. Materials and Methods

2.1. Genetic Materials and Planting

One thirty rice genotypes were used for this study under irrigation system. Among the genotypes, 123 were collected from KAFACI in South Korea, three from University of Port Harcourt, one from NCRI and three from AfricaRice-Nigeria (Table 1). Rice seeds were sown in the nursery and 21 d old seedlings were transplanted into the field. The experiment was laid out in 26 × 5 Alpha Lattice Design at 20 cm × 20 cm spacing in two replications.

Recommended cultural practices such fertilizer application and weeding were adopted. At transplanting day 200 kgha^{-1} of NPK (15-15-15) was applied as basal and 100 kgha^{-1} of Urea in two splits of 65kgha-1 at mid-tillering stage (30 DAP) and 35 kgha-1 at booting stage (45 DAP). Weeds were controlled twice by selective herbicide Oriozol Plus (300 ml/ 20 liters of water) at early stage of crop development and before flowering.

2.2. DNA extraction and SSR Analysis

DNA was extracted from the leaves of 25 d old seedlings according to the CTAB modified method. The obtained DNA was dissolved in 100 μl of 1X TE buffer and stored at -20°C, and then diluted to 25 ng/μl using double distilled water to obtain the working solution.

A total of 17 single sequence repeart (SSR) were used to evaluate the polymorphism of 130 genotypes (Table 2). PCR was performed in 10 μl reaction mixture volume containing 2 μl of DNA, 6.49 μl of sterile H$_2$O, 1 μl of 10X buffer, 0.2 μl of dNTPs (1 mM), 0.75 μl of MgCl$_2$ (50 mM), 0.1 μl of Taq polymerase, 0.5 μl of the Forward primer (100 mM) and 0.5 μl of the Reverse primer (100 mM). Mineral oil was added to the reaction mixture to prevent evaporation. The PCR reaction was performed using a thermal cycler system (96 well alpha-units). The PCR program used consisted of an initial denaturation at 94°C for 2 min followed by a series of 35 cycles, each of which consisted of a denaturation at 94°C for 30 second, annealing at 55°C for 45 second and extension at 72°C for 45 second. The program ends with a final elongation of 72°C for 2 min. The SSR products were separated on a denaturing 6% polyacrylamide gel and visualized by silver staining. The electrophoretic bands were scored as 1 (present) or 0 (absent) to form a raw data matrix for further analysis.

Table 1. *Rice genotypes used in ths study under irrigation condition from October to March at Africa Rice experiment station-IITA.*

Sources	Genotypes					
	UPN334	UPN339	UPN238	UPN289	UPN237	UPN307
	UPN335	UPN343	UPN280	UPN248	UPN274	UPN249
	UPN346	UPN316	UPN227	UPN326	UPN328	UPN243
	UPN315	UPN234	UPN268	UPN311	UPN327	UPN347
	UPN348	UPN259	UPN246	UPN245	UPN239	UPN336
	UPN230	UPN296	UPN275	UPN231	UPN341	UPN294
South Korea	UPN349	UPN288	UPN272	UPN251	UPN266	UPN340
	UPN241	UPN287	UPN308	UPN233	UPN309	UPN269
	UPN271	UPN333	UPN244	UPN320	UPN312	UPN293
	UPN270	UPN319	UPN262	UPN317	UPN282	UPN229
	UPN304	UPN276	UPN279	UPN247	UPN323	UPN345
	UPN252	UPN292	UPN314	UPN281	UPN299	UPN232
	UPN290	UPN286	UPN325	UPN329	UPN295	UPN344
	UPN264	UPN321	UPN297	UPN310	UPN322	UPN260
	UPN250	UPN261	UPN240	UPN267	UPN324	UPN285
	UPN306	UPN300	UPN332	UPN253	UPN337	UPN273
	UPN228	UPN331	UPN306	UPN300	UPN332	UPN253
	UPN330	UPN255	UPN277	UPN247	UPN235	UPN284
	UPN283	UPN291	UPN242	UPN258	UPN342	UPN256
	UPN265	UPN303	UPN278	UPN301	UPN305	UPN338
	UPN313	UPN257	UPN298	UPN337	UPN273	UPN228
	UPN301	UPN305	UPN265	UPN303	UPN278	UPN331

Sources	Genotypes					
	UPN236	UPN263	UPN298	UPN257	UPN302	UPN318
University of Port Harcourt	UPIA3	UPIA2	UPIA1			
Africa Rice	SAHEL210	SAHEL134				
NCRI	FARO44					
Africa Rice-Benin	ARICA3					

2.3. Data Analysis

Analysis of variance for each character was done by using genotypes as random effects and replication as fixed effect using the Mixed Procedure "proc mixed" in SAS system for windows version 9.2. Genetic parameters were estimated to identify genetic variation among accessions and to determine genetic and environmental effects on various characters. These genetic parameters were calculated [13, 14].

Genotypic variance

$$\theta g^2 = (MSG-MSE)/r \tag{1}$$

Where MSG is the mean square of genotypes, MSE is mean square of error, and r is number of replications

Phenotypic variance:

$$\theta_p^2 = \theta_g^2 + \theta_e^2 \tag{2}$$

Where $\sigma^2{}_g$ is the genotypic variance and $\sigma_e{}^2$ is the mean squares of error.

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variance (PCV):

$$GCV = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100 \tag{3}$$

$$PCV = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100 \tag{4}$$

Where $\sigma^2 g$ = Genotypic variance,
$\sigma^2 p$ = Phenotypic variance

GCV and PCV values were categorized as low (0 10%), moderate (10-20%) and high (20% and above).

Heritability (Broad sense) was computed manually [15].

$$H^2 = \frac{\sigma^2 g}{\sigma^2 P} \times 100 \tag{5}$$

Where $\sigma_g{}^2$ is the genotypic variance and $\sigma_\rho{}^2$ is the phenotypic variance

The Heritability was categorized as low (0-30%), moderate (30-60%) and high (60% and above) as [16].

Expected genetic advance (GA):

$$GA = k \times \sigma_p \times H^2{}_b \tag{6}$$

Where, $H^2{}_b$ = Heritability in broad sense, σp = Phenotypic standard deviation, GA = Expected genetic advance and k = the standardize selection differential at 5% selection intensity (K = 2.063)

Genetic advance expressed as a percentage over mean (GAM) is calculated as:

$$GAM = \frac{GA}{\bar{x}} \times 100 \tag{7}$$

Where, GAis the genetic advance and $\bar{x}$ is the general mean of the traits.

Pearson's correlation coefficients were computed to evaluate the relationship among the observed variables and the data analyzed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm and dendrogram was constructed based on similarity groups using Ward method [17]. Genetic relationships were also calculated and a dendrogram derived to show the morphological relatedness among genotypes under study. Principal component analysis was performed using correlation matrix to examine the percentage contribution of each trait to total genetic variation by the software SAS version 9.2.'

Powermarker 3.25 was used to calculate number of alleles per locus, major allele frequency, and expected heterozygosity [18]. Polymorphism information content (PIC) was calculated using the following formula [19].

$$PIC = 1-\Sigma X^2{}_k/n \tag{8}$$

Where: $X^2{}_k$ = frequency of the kth allele in the set of genotypes investigated, n = number of genotypes.

***Table 2.** Sequence of primer pairs/markers used in this study during dry season at IITA Bioscience Laboratory.*

Primer name	Forward Primer	Reverse Primer	Tm
RM 587	ACG CGA ACA AAT TAA CAG CC	CTT TGC TAC CAG TAG ATC CAG C	55
RM 341	CAA GAA ACC TCA ATC CGA GC	CTC CTC CCG ATC CCA ATC	55
RM 7000	CCC TTC TTT TCA ACT GAA TA	TTG TAA CAA TGA ACT CGT TC	55
RM 7389	AGC GAC GGA TGC ATG ATC	TTG AGC CGG AGG TAG TCT TG	55
RM 163	ATC CAT GTG CGC CTT TAT GAG GA	CGC TAC CTC CTT CAC TTA CTA GT	55
RM 510	AAC CGG ATT AGT TTC TCG CC	TGA GGA CGA CGA GCA GAT TC	55
RM 6775	GCA GAT CAA GTA TGC CTG CC	TCG CTA GAT AGG GGA TGT GG	55
RM 3183	GCT CCA CAG AAA AGC AAA GC	TGC AAC AGT AGC TGT AGC CG	55
RM 1370	AAA CGA GAA CCA ACC GAC AC	GGA GGG AGG AAT GGG TAC AC	55
RM 340	GGT AAA TGG ACA ATC CTA TGG C	GAC AAA TAT AAG GGC AGT GTG C	55
RM 336	CTT ACA GAG AA CGG CAT CG	GCT GGT TTG TTT CAG GTT CG	55
RM 588	GTT GCT CTG CCT CAC TCT TG	AAC GAG CCA ACG AAG CAG	55
RM 279	GCG GGA GAG GGA TCT CCT	GGC TAG GAG TTA ACC TCG CG	55

Primer name	Forward Primer	Reverse Primer	Tm
RM 3805	AGA GGA AGA AGC CAA GGA GG	CAT CAA CGT ACC AAC CAT GG	55
RM 402	GAG CCA TGG AAA GAT GCA TG	TCA GCT GGC CTA TGA CAA TG	55
RM 14001	TGT GGC TGG GCT CCG ATA CC	ACC CTG CAG GAT CAT CAG AAC G	55
RM 1369	AAC CTG AGA GTG CCA ATT GG	TCC CCT ATG AAA GCG GAT TC	55

Tm – Annealing temperature (°C).

UPGMA dendrogram was constructed based on Neighbor Joining (NJ) method in DARwin 6.0.12 showing genetic relationships among 130 rice genotypes.

3. Results

3.1. PCV, GCV, Genetic Advance and Heritability of Estimated Traits

The study revealed higher phenotypic variation for all the traits measured than genotypic variance (Table 3). The PCV estimates were higher in all traits than the corresponding GCV. The GCV and PCV were low for DM although they were moderate for SpFert and 1000-GrW (Table 3). Low GCV was recorded for PH whereas PCV showed high value for the same traits. Moderate high GCV was observed for PaW, NSp and grain yield.

Table 3. *Estimates of genetic parameters, broad sense heritability, and genetic advance 12 morphological traits of 130 rices genotypes evaluated at Africa rice/IITA-Ibadan, Nigeria.*

Traits	σ_g^2	σ_e^2	σ_ρ^2	GCV (%)	PCV (%)	H² (%)	GA (%)
DFLW	65.88	10.67	76.55	8.95	9.65	86.06	17.12
DM	96.93	5.89	102.82	8.05	8.29	94.27	16.11
PH	96.91	375.78	472.69	9.74	21.52	19.45	8.62
Till	0.37	1.05	1.42	8.3	16.27	26.05	8.73
PL	6.68	2.87	9.55	9.94	11.88	69.94	17.12
NPrTill	0.41	0.95	1.36	9.14	16.65	30.14	10.34
PaW	0.4	0.56	0.96	18.17	28.15	41.66	24.16
FillGr	585.51	1009.51	1595.02	19.46	32.11	36.7	27.56
SpFert (%)	7174.00	46.49	118.23	10.66	13.69	60.67	17.1
NSp	873.00	1770.37	2643.37	18.87	32.85	33.02	22.34
1000-GW	8.44	1.43	9.87	11.62	12.55	85.51	22.13
GY (kg/ha)	271408.68	161042.6	432451.3	18.24	23.02	62.76	29.76

σ_g^2 = genotypic variance; σ_e^2 = error variance, σ_ρ^2 = Phenotypic variance; GCV-Genotypic coefficient of variation; PCV- Phenotypic coefficient of variation; H²- Broad sense heritability; GA- Genetic advance; DFLW = Days to flowering, DM = Days to maturity, PH = Plant height, NTill = No of tillers/plant, PL = Panicle length, NPrTill = No of productive tillers/plant, PaW = Panicle weight, Fill Gr = No of filled grain/spikelets, SpFert (%) = Spikelets fertility, NSp = No of spikelets/panicle, 1000GrW = 1000 grain weight, GrY = Grain yield (kg/ha).
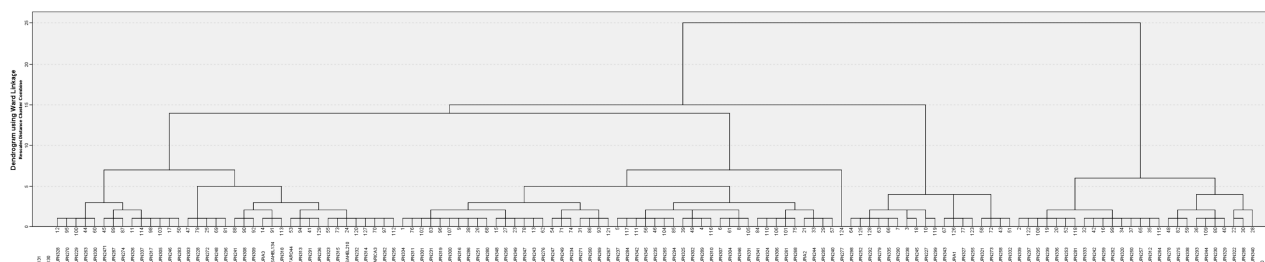
Figure 1. *Dendogram using agglomerative clustering method representing distribution of 130 rice genotypes based on morphological traits (from left of the figure is cluster 1 and sub-cluster 1a and 1b; cluster 2 and sub-cluster 2a and 2b; cluster 3 and cluster 4 and sub-cluster 4a and 4b, respectively).*

3.2. Cluster and Principal Components Analysis of Rice Genotypes Evaluated

Hierarchical cluster analysis of some morphological traits and yield are depicted in the Figure 3. Four main clusters (1, 2, 3, 4) were identified at 12.5 genetic similarity index. Cluster 1 recorded 37 genotypes with 2 sub-clusters: 1a and 1b. Genotypes of cluster 1 had good agronomic traits such as number of productive tiller (UPN296). Cluster 2 had 48 genotypes with two sub-clusters. The major sub-cluster 2a was differentiated at 5% genetic similarity index with 47 genotypes, while sub-cluster 2b consisted only one genotype (UPN277) which is the most diversified among the genotypes.

Sub-cluster 2a includes genotypes which had characters such as high grain yield (UPN347, UPN266, UPN268 and UPIA2), panicle weight and number of filled grains. Grouping of these genotypes in one cluster indicated their parental relationships. Cluster 3 recorded minimum genotypes (18) which are superior for plant height, 1000-grain weight, panicle length an panicle weight (UPN335, UPN227, UPN327 and UPN332). Cluster 4 consisted 26 genotypes with 2 sub-clusters. Genotype UPN340 is very short durated and much earlier then the other genotypes.

Principal component analy sis indicates that the first four components accounted for 73.59% of total variation (Table 4). It also revealed that the first principal component accounted

for 31.12% of total variance. The second component accounted for 16.20% of the total variation and featured 1000-grain weight as the principal trait, followed by days to 50% flowering (0.36), day to maturity (0.36) and number of spikelets (-0.35) in negative direction. The third principal component accounted for 16.07% of the total variation and it was loaded mainly by traits such as number of tillers per plant (0.67). The fourth principal component accounted for 10.20% of the total variance. The variables that contributed negatively are day to 50% flowering (-47), day to maturity (-0.41), number of filled grain (-0.39) and spikelets fertility (-0.35), while panicle length (0.52) contributed positively (Table 4).

Table 4. Eigenvalues, % variance and cumulative% variance of 12 morphological traits for the first four principal components.

Traits	PC1	PC2	PC3	PC4
Eigen value	3.73	1.94	1.92	1.22
%Variance	31.12	16.20	16.07	10.20
Cumulative variance%	31.12	47.32	63.39	73.60
50% Day to flowering	0.30	0.36	0.11	-0.47
Day to maturity	0.31	0.36	0.08	-0.41
Plant height	0.39	0.22	0.03	0.25
No. of tillers	-0.05	-0.00	0.67	0.14
Panicle Length	0.26	0.08	-0.05	0.52
No. of Prod Till	-0.08	0.01	0.68	0.08
Panicle Weight	0.44	-0.10	0.02	0.10
Spikelets Fertility	0.17	-0.25	0.03	-0.35
No. of Filled Grain	0.39	-0.41	-0.00	-0.39
1000 Grains Weight	0.19	0.48	-0.05	0.29
Grain Yield	0.17	-0.24	0.22	0.07

3.3. Pearson Correlation Analysis Among Morphological Traits

The correlation analysis was done on the studied genotypes using SAS software version 9.2 (Table 5). It reveales that days to 50% flowering showed positive and highly significant correlation with days to maturity and 1000-grain weight and positive significant correlation with number of spikelets, spikelets fertility, panicle weight, plant height and yield. Days to maturity was highly significant positively correlated with panicle length. Plant height showed significant correlation with the panicle length. Panicles weight was highly significant positively correlated with number of filled grains. Number tillers per plant was highly significant positively correlated with number of productives tillers (Table 5).

Table 5. Pearson correlation analysis of 12 morphological traits to characterize 130 rice genotypes.

Traits	DFLW	DM	PH	NT	PL	NPT	PW	NFG	SpFert (%)	NS	1000-GW
DM	0.905***										
PH	0.461**	0.431**									
NT	0.356**	0.409**	0.161								
PL	0.549**	0.682***	0.422**	0.272**							
NPT	0.351**	0.391**	0.151	0.937**	0.214						
PW	0.371**	0.356**	0.276**	0.165	0.381**	0.16					
NFG	0.271**	0.281**	0.198	0.082	0.234	0.06	0.717***				
SpFert (%)	0.505**	0.547**	0.275**	0.211	0.433**	0.232	0.388**	0.417**			
NS	0.259**	0.287**	0.191	0.094	0.228	0.055	0.656***	0.938***	0.146		
1000GW	0.628***	0.659**	0.385**	0.247**	0.569**	0.240**	0.360**	0.084	0.259**	0.124	
GY	0.285**	0.317**	0.184	0.332**	0.311**	0.289**	0.322**	0.333**	0.335**	0.284**	0.172

DFLW = Days to flowering, DM = Days to maturity, PH = Plant height, NT = No of tillers/plant, PL = Panicle length, NPT = No of productive tillers/plant, PW = Panicle weight, NFG = No of filled grain/spikelets, SpFert (%) = Spikelets fertility, NS = No of spikelets/panicle, 1000GW = 1000 grain weight, GY = Grain yield (kg/ha).

Highly significant positive correlation with panicles weight and number of filled grain per panicle while non-significant positive correlation with spikelet fertility and number of spikelets. Yield showed significant positive correlation with all the studied traits except plant height and 1000-grain weight. (Table 5)

3.4. Genotyping Using SSR Molecular Markers

Total of 17 SSR primers were found to be polymorphic (Figure 2) and 70 alleles were identified. The number of alleles ranged from 2 (RM587) to 6 (RM336) with an average of 4.12 alleles per primer (Table 6 and Figure 2).

Genetic diversity: The highest level of gene diversity value (0.79) was observed in loci RM336, and RM588, and the lowest level of gene diversity value (0.31) was observed in loci RM3183 with a mean diversity of 0.58 (Table 6 and Figure 2). The genetic diversity of each SSR marker appeared to be associated with the number of alleles detected.

PIC values: As a measure of the informativeness of each marker, PIC value ranged from a low of 0.26 (RM31830) to a high of 0.76 (RM336, RM588) with a average of 0.53 (Table

6 and Figure 2). The average heterozygosity value (0.08) is very low as expected for self-pollinated crop.
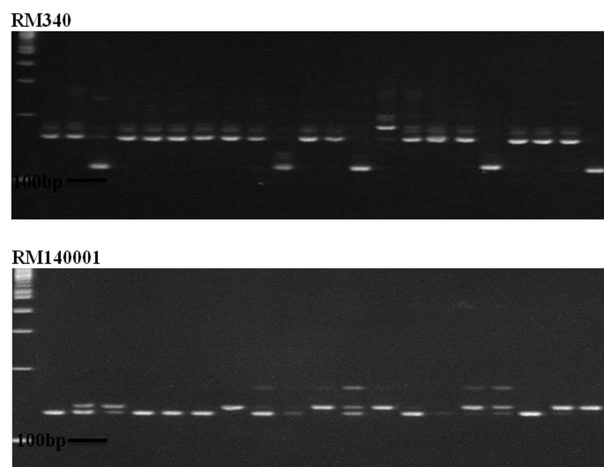
RM340

RM140001

Figure 2. *Allelic variations of some rice genotypes generated by SSR markers RM340 and RM 14001 observed on 6% PAGE. M: 100 bp DNA ladder.*

Table 6. *Data on major allele frequency, allele number, gene diversity, heterozygosity and PIC obtained among 130 rice genotypes for 17 SSR markers.*

SSR Markers	Ma	Na	GD	He	PIC
RM340	0.72	5	0.45	0.05	0.42
RM336	0.25	6	0.79	0.07	0.76
RM341	0.34	5	0.73	0.14	0.68

SSR Markers	Ma	Na	GD	He	PIC
RM1369	0.51	5	0.62	0.06	0.56
RM163	0.5	5	0.68	0.06	0.64
RM279	0.7	4	0.45	0.04	0.39
RM402	0.52	3	0.51	0.04	0.39
RM510	0.68	3	0.44	0.09	0.35
RM587	0.68	2	0.43	0.17	0.34
RM588	0.28	6	0.79	0.12	0.76
RM1370	0.75	3	0.39	0.07	0.35
RM3183	0.81	2	0.31	0.11	0.26
RM6775	0.56	3	0.55	0.13	0.45
RM7000	0.3	5	0.77	0.05	0.74
RM7389	0.47	3	0.62	0.03	0.54
RM3805	0.51	5	0.66	0.11	0.62
RM14001	0.31	5	0.74	0.08	0.69
Mean	0.52	4.12	0.58	0.08	0.53

3.5. Clustering of Genotypes Found in Molecular Diversity Analysis

The genetic distance among rice germplasm led to the construction of Neighbor-Joining (NJ) tree as shown in Figure 3. The dendrogram revealed three main clusters (1, 2, 3) in the rice genotypes studied at 51.8 genetic similarity index. Cluster 1 comprised 34 genotypes with only one African line (UPIA1) among Korea accessions. Cluster 2 consisted of 29 genotypes while cluster 3 had 57 genotypes, with 3 sub-clusters 3a, 3b and 3c. The cluster 3a and cluster 2 comprised only Korea lines, while clusters 1 and sub-cluster 3b and 3c are mixture of Korea lines and African adapted varieties (Figure 3)
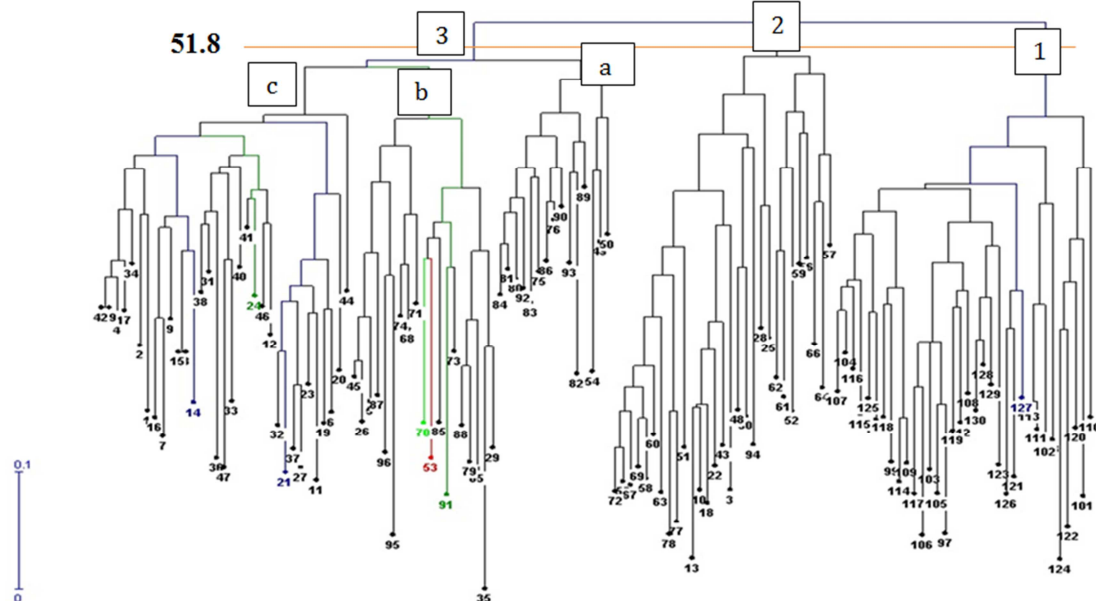
Figure 3. *UPGMA dendogram based on Nei's (1972) genetic distance, summarizing the data on differentiation among 130 rice genotypes according to SSRs marker analysis.*

4. Discussion

4.1. PCV, GCV, Genetic Advance and Heritability of Estimated Traits

Phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were categorized as low (0-10%), moderate (10-20%) and high (>20%) as indicated by Sivasubramanian and Madhavamenon, (2012). Therefore, high PCV was recorded for number of spikelets, number of filled grains, panicle weight, grain yield and plant height and moderate GCV for number of filled grains, number of

spikelets, grain yield and panicle weigh, which suggests the possibility of improving this trait through hybridization to create variation before selection. The estimates of GCV and PCV were low in the present study for days to maturity (8.05%, 8.29%). Similar findings were reported [20]. In general, a high coefficient of variability indicates good level of selection and improvement of these traits. Low values indicate the need for creation of variability either by hybridization or mutation followed by selection [21].

The magnitude of phenotypic coefficient of variation (PCV) estimates in the present study was found to be slightly higher than their respective genotypic coefficient of variations (GCV) for all the studied characters which might be due to environmental factors in rice [22, 23]. However, the narrow magnitude of difference between phenotypic and genotypic coefficients of variations were recorded for characters such as days to maturity, plant height, and panicle length indicating limited influence of environment in the expression of these characters. These results corroborate with [24], thus, selection based on phenotypic performance of these characters would be effective to bring about considerable genetic improvement. Therefore, the rice breeders may make superior genotypes selection based on phenotypic performance for these traits. High heritability in the broad sense recorded for day to maturity, days to 50% flowering, 1000 grain weight, panicles length and grain yield shows that these traits can be easily selected phenotypically in the field during a breeding programme. Panicles weight, number of filled grains, number of spikelets and number of productive tillers had medium heritability estimates, which indicates that improvement can be made through simple selection. In contrast, plant height, number of tillers and number of productive tillers had low heritability which indicates greater influence of environment on the expression of the characters, direct selection for these traits may not be effective. Therefore, methods of selection based on progeny testing and families are more effective and efficient. Similar results were also reported by other researchers in rice [23, 25, 26] for number of productive tillers per plant. Knowledge of genotypic coefficient of variations coupled with heritability estimates and genetic advance could be most useful. Genetic Advance (GA) ranged from 8.62% for plant height at maturity to 29.76% for yield. This suggests that genetic improvement of these traits can be achieved through mass selection [27]. Traits such as plant height, tiller number, and number of spikelets which exhibited low heritability estimates, are likely to be more influenced by the environment and selection by progeny testing or in segregating population could be preferred. High heritability estimates and genetic advance had been earlier reported in crops [28, 29, 30]. Previous studies suggested that high broad sense heritability; PCV and GCV for yield and yield related traits are good predictors for high grain yield in crops [31].

4.2. Cluster and Principal Component Analysis

Dendrogram constructed on the Euclidian distance matrix using Ward's linkage method grouped the 130 lines of rice into four clusters at genetic dissimilarity level of 12.5 (Figure 3.). Cluster analysis provided with a complete view of genetic variation among 130 genotypes. This is a useful tool in a hand of the plant breeders for the genetic improvement of rice. Sub-cluster 1b, 2a and cluster 3 are composed of Korea and African lines; however sub-cluster 1a, 2b and cluster 4 consists only of Korea lines. If we select one genotype from each cluster then UPN296, UPN347, UPN277, UPN332 and UPN340 could be selected from cluster 1, cluster 2a, cluster 2b, cluster 3 and cluster 4 respectively for breeding poposes. For future breeding program, parents are selected from diverged clusters with better agronomic performance or from each cluster one parent is selected and then the selected genotypes are subjected to crossing therefore, UPN340 could be useful in breeding for shorten duration rice [32],

Principal Components Analysis (PCA) explained the genetic variation among the genotypes for all agro-morphological traits under study. Data was considered in each component with Eigenvalues more than 1, which determines as a minimum 10% of the variation [33]. Superior Eigenvalues are considered as best attributes in principal components. In our study four principal axis exhibited Eigenvalues of >1 and showed cumulative variation of 73.59%. It indicates that the identified characters within these components showed immense influence on the phenotype of the genotypes.

PCA explained the genetic diversity of the rice germplasm. It assesses the importance and the contribution of each coefficient of correct vectors indicates the degree of contribution of every original character with each principal component is correlated and that the higher the coefficients, the more effective they will be in discriminating between germplasm, regardless of the positive or negative sign. [34, 35] In this present study variation did exist among 130 rice genotypes with respect to the 12 traits that were evaluated, forinstance, in PC4 (Principal Component 4), panicle length, 50% days to flowering and day to maturity and panicle length in PC4 and PC4 in grain yield and other related traits [36, 37]. The PC1 (Principal Component 1) consisted of plant eight, panicle weight, number of filled grains, and number of spikelets, this corroborate [38]. Principal component analysis revealed that PC1 and PC2 were dominated by yield related traits viz. 1000-grain weight, panicle length, plant height, panicle weight, spikelet fertility, number of spikelets. These traits are considered as the most important for classifying the variation existing in the germplasms. Therefore, intensive selection procedures could be designed to bring about quick improvement of dependent variable such as grain yield by choosing lines from PC1 and PC2. Recent report indicated that in PC2 plant height had high weight and factor loading, thus indicates that PC2 represents latent variables for morphological traits and characters with high variability are expected to provide high level of gene transfer during breeding programs [39-41].

4.3. Correlation Analysis

The relationships existing between 12 quantitative traits as shown by correlation coefficients (Table 3). Grain yield was positively correlated with panicle weight, spikelets fertility, number of spikelets per panicle, panicle length, number of tillers per plant, number of productive tillers per plant and number of filled grain. The degree of association among characters is an important factor when dealing with a complex trait such as yield that is controlled by many genes and highly influenced by the environment [42]. The significant positive correlations between yield and some of the traits indications that selection in favour of these traits could lead to positive indirect selection for grain yield improvement. High positive association was observed between 1000-grain weight and rice grain yield. Also, high positive correlation was reported between plant height, productive tillers per plant and grain yield [39, 43].

4.4. Genotyping Using SSR Molecular Markers and Clustering of the Genotypes

All the 17 SSRs primers were found to be polymorphic (photo 1) and 70 alleles were identified. The frequency of major allele at each locus varied from 0.25 to 0.81 with average of 0.52. The number of alleles ranged from 2 (RM587) to 6 (RM336) with an average of 4.12 alleles per primer (Table 6), this number is similar to the average value of 4.3 alleles per locus and found up to 203 alleles for 48 SSR among 11 Venezuelan rice cultivars, thus indicates markers used were less polymorphic as compared to this study [44].

Furthermore, the average value (4.12) of alleles per locus obtained was higher than the values reported in previous studies [44, 45]. In this study, maximum number of polymorphic alleles (6) was obtained with the marker RM336 and RM588, while the minimum number of polymorphic alleles (2) was obtained with RM587 and the average heterozygosity value (0.08) is very low as expected for self-pollinated crop as rice.

PIC value is a reflection of allele diversity and frequency among the genotypes. It was calculated for each locus to evaluate the information of each marker. In this study, PIC value ranged from 0.26 (RM3183) to 0.76 (RM336) with a mean of 0.53 were obtained. The highest PIC value 0.76 was obtained for RM336 and RM588 followed respectively by RM7000 (0.74) and RM14001 (0.69). The primers RM336 and RM588 were considered as the best markers.

Markers with a PIC value of 0.5 or higher indicate that they are highly informative and extremely useful in distinguish the polymorphism rate of a marker at a specific locus [46]. The PIC value found in this study is similar to previous [47, 48]. Though, PIC value lower than 0.5 this may hide information carried by gene at a specific locus [45, 49]. The genetic diversity of each SSR marker appeared to be associated with the number of alleles detected per locus that is the higher PIC value of a locus, the higher the number of alleles detected [50].

4.5. Clustering of Genotypes Found in Molecular Diversity Analysis

Similarities were observed in morphological and moleculaer clustering of the genotypes evalauated. The genetic distance among rice germplasm led to the construction of Neighbor-Joining (NJ) tree as depicted in Figure 2. The dendrogram revealed three main clusters (1, 2, 3) in the rice genotypes studied at 51.8 genetic similarity. Basically, crossing of genotypes of the same cluster may not to give superior hybrids or segregants, because of the little divergence that will be observed among them. However, a larger divergence will generate high amount of heterosis in F1 and subsequent generations. Cluster 1 comprised 34 genotypes with only one African line (UPIA1) among Korea accessions. Cluster 2 consisted of 29 genotypes all Korea, and cluster 3, the maximum with 67 genotypes. It divided into 3 sub-clusters 3a, 3b and 3c. Cluster 3a comprised of only Korea lines, while clusters 3b and 3c are mixtures of Korea lines and African adapted varieties, thus crosses between clusters could create higher heterosis than the parentallnes. The dendrogram showed that African adapted varieties were closely related to some of Korea lines belonging to the same cluster, while UPIA1 belonging to different cluster suggesting that these variety is genetically diverse in origin. This may be of help to rice breeders in the selection of parental lines for further hybridization and genetic improvement.

5. Conclusion

Results revealed that PCV was higher than GCV for all the traits under studied, which could be due to environmental factors for the expression of these characters. Breeders should take this into consideration when setting a breeding programme for yield improvement. Yield was positive significant correlated with all traits under studied except plant height ant 1000-grain weight. The first four components of PCA accounted for 73.59% of total variation giving an estimation of the relative contribution of various traits to the genotypes under studied. The genotypes were grouped into three main group for molecular and four for morphology dendrogram. Crosses between these divergent clusters may to produce high heterosis in F1 generation. The PIC values ranged from a low of 0.26 (RM3183) to a high of 0.76 (RM336, RM588) and averaged 0.53 indicating highly informative and extremely useful in distinguish the polymorphism rate of a marker at a specific locus. High genetic diversity observed between cluster 1 and cluster 3 indicate that genotypes from cluster 1 in association with those from cluster 3 could be good candidate in breeding programme for genetic improvement. These genotypes UPN347, UPN277, UPN296, UPN255 and UPIA2 shown to be the most promising genotypes that could be used for rice hybridization, genetic improvement and rice hybrid programme. UPN340 could be used in breeding for shorten duration rice variety.

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