African Journal of Biotechnology Vol. 4 (11), pp. 1234-1244, November, 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Full Length Research Paper

Phylogenetic diversity and relationships among 40 rice accessions using morphological and RAPDs techniques

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Accepted 15 June, 2005

Morphological characterization of 40 rice accessions using 14 agro-botanical traits was done in a field experiment in an augmented randomized complete block design. The aim of the work was to study variations and to select lines that can be used as potential parents in a future breeding program. The single linkage clustering, the Principal Components Axes and a morphological dendrogram were used to group the accessions. Genetic relatedness among accessions based on random amplified polymorphic DNA (RAPD) molecular marker data was also presented in form of a dendrogram using the Unweighted Pair Group Method with Arithmetic mean (UPGMA). Relative effectiveness of the RAPD markers and genetic diversity among accessions as revealed by botanical descriptors were compared. The single linkage cluster technique classified the 40 accessions into six morphological groups whereas the PCA re-ordered the accessions into four broad groups that had within cluster similarities and inter-cluster morphological variations. RAPDs were highly polymorphic, more discriminatory and informative as they were able to differentiate more pairs of accessions than the botanical descriptors. IITA rice accessions TOX 3052-46-3-3-2-1 and TOX 3027-44-1-E4-2-2 and Brazilian accessions (CL SELECCION 3B and 450) that performed better than checks could be selected for a future breeding program.

Key words: Accessions, morphological, phylogenetic diversity, polymorphism RAPD, rice, UPGMA.

INTRODUCTION

Rice (*Oryza sativa* L.) constitutes a principal source of calories in Africa; it is a staple food crop in Côte d'Ivoire, Gambia, Guinea, Guinea-Bissau, Liberia, Madagascar, Mauritania, Senegal and Sierra Leone, Nigeria, among others (Malton, et al., 1998). Its adoption as a principal staple food is increasingly spreading to other parts of Africa; however, self sufficiency in rice production is declining as demand increases. Rice is no longer a luxury food but has become the cereal that constitutes a major source of calories for the urban and rural poor. There has been tremendous increase in the import of the commodity because production has not met with demand (WARDA, 2000). Hence there is an urgent need to increase and improve the production of rice in Africa, in order to meet up with the high demand. The need for expansion of rice cultivation does not only depend on cultural practices and

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Table 1. Pedigree listing and origin of the 40 rice accessions.

Entry no	Designation	Origin
1	AFAAMWANZA 2-200	TANZANIA
2	ANDRAMONTA 3	MADAGASCAR
3	B4000J-KP-9-3	INDONESIA
4	B4354G-PN-75	INDONESIA
5	B5332-13D-MR-1-1	INDONESIA
6	BKN 6806-46-60	THAILAND
7	BR 51-282-8-HR83	BANGLADESH
8	BR 51-315-4	BANGLADESH
9	BW 306-8	SRI LANKA
10	BW 311-7	SRI LANKA
11	CHANZI (BEIRA)	MOZAMBIQUE
12	CHEIRO V (NANIALO)	MOZAMBIQUE
13	CL SELECCION 3B	BRAZIL
14	CL SELECCION 450	BRAZIL
15	CN 836-3-10	INDIA
16	FACAGRO 59	BURUNDI
17	FAROX 318-1-3	NIGERIA
18	FOFIFA 47	MADAGASCAR
19	FOTSIFOTSY 156A	MADAGASCAR
20	IET 8113	INDIA
21	IET 8565	INDIA
22	IR 25077-KKN-38-PM-1-2-1	IRRI
23	IR 37096-50-1-3-3	IRRI
24	IR 41111-13-1-1-2	IRRI
25	IR 42221-2-2-3-1	IRRI
26	IR 42241-76-2-2-2	IRRI
27	IR 43450-SKN-506-2-2-1	IRRI
28	IR 45411-40-2-1	IRRI
29	IR 46375-CPA-19-3-1	IRRI
30	IR 48725-B-B-129-1	IRRI
31	ITA 222 (Check)	IITA
32	ITA 324	IITA
33	ITA 368 (Check)	IITA
34	TCS 10	MOZAMBIQUE
35	TOX 3027-44-1-E4-2-2	IITA
36	TOX 3052-46-3-3-2-1	IITA
37	TOX 3081-36-2-3	IITA
38	TOX 3093-4-5-3-3	IITA
39	TOX 3098-4-2-1-2	IITA
40	TOX 3100-32-2-1-3	IITA

management, but also on the suitability of rice varieties, which must be drawn from existing germplasm that has been collected and conserved by genetic resources centers (Ng et al., 1988).

Evaluation and characterization of potential varieties should form an important constituent of these collection efforts because of their in-built genetic variability due to several generations of growing and selection by breeders and farmers. However, the utilization of these rice genetic resources had been limited to only adaptable genotypes (Caldo et al., 1996). As a result, the diversity of these genetic resources is being lost to the need for higher yields and early maturity. However a successful breeding programme will depend on the genetic diversity of a crop for achieving the goals of improving the crop and producing high yielding and better resistant varieties (Padulosi, 1993). Therefore, there is the need to diversify the genetic base of improved rice varieties, and the first step towards this is to evaluate and characterise available rice germplasm or genotypes at both the morphological and molecular levels. This is because the evaluation of phenotypic diversity usually reveals important traits of interest to plant breeders (Singh, 1989). Despite the importance of morphological characterization and the data produced by this process, very little work has been done on the evaluation and characterization of rice germplasm available in Nigeria. The main objective of this study is to analyse the morphological and molecular organisation of the existing diversity in the potential rice germplasm as suitable donor parents in a breeding program in Nigeria. The specific objectives are to compare genetic relatedness among the various genotypes and analyse rice biodiversity and yielding ability.

MATERIALS AND METHODS

Evaluation of morphological traits

The rice germplasm lines used in this study consisting of 38 accessions and two different checks (ITA 222 and ITA 368) were collected from the Genetic Resources Unit of the Africa Rice Center (WARDA) Table 1. The materials were selected based on variations in potential yields and number of tillers among accessions regenerated by the Genetic Resources Unit of WARDA in 2003 rainy season at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The experimental work was conducted under rainfed lowland conditions during the 2003 dry season at IITA, Ibadan, Nigeria. The augmented experimental design introduced by Federer (1956, 1961, 1991) was used for the experiment. An augmented experimental design (AED) is usually useful for testing a large number of genotypes in early generations when valid statistical analyses are needed particularly when seed supplies are too limited to permit replication. The basic concept of augmented design construction is to establish a standard replicated design using checks for which sufficient seeds are available. Each replicate forms a complete block, incomplete block, or cell, depending on the standard design. Additional unassigned plots are created within each replicate and unreplicated entries for which there are insufficient seeds. Then entries are assigned to these plots in the form of an incomplete block design. The seeds were sown in the nursery bed in July, 2003 before they were transplanted at 21-day old with a spacing of 20 cm x 20 cm. One seedling was transplanted per hill and the inter-plot spacing was 40 cm. A plot size of 0.6 m x 5 m with 4 rows was used for each accession in the field.

Table 2. List of characters studied in the experiment.

Character	Abbreviation
Grain yield	GY
Flowering date	FD
Maturity date	MD
Plant Height	PHT
Total Tiller	TT
Filled Tiller	FT
Tiller diameter	TD
Leaf length	LL
Leaf width	LW
Panicle length	PL
Panicle shattering	PS
Grain length	GL
Grain width	GW
100-GWT	GWT

Table 3. Mean squares from analysis of variance for augmented randomized complete block design for fourteen traits measured in 40 rice accessions.

Source	df	Mean Square						
		Yield	Flow date	Mat.date	Plant height	Total tiller	Filled Tiller	Tiller dm
Block	2	1648.67	$6.39 \times 10^{28**}$	$6.38 \times 10^{28**}$	$3.19 \times 10^{-28**}$	0.50	0.17	0.00
Check(c)	1	16,914.00**	841.08**	952.04**	2510.94**	77.95**	68.45**	4.69**
X (c)	37	51,005.00**	39.54**	91.20**	225.39**	10.94*	9.60*	0.40**
Error	3	1391.17	0.12	0.16	6.01	0.17	0.05	0.01
Total	43							

Table 3. contd.

Source	df	Mean Square						
		Leaf length	Leaf width	Pan length	Pan shatt	Grain length	Grain width	100-gwt
Block	2	0.27	$3.21 \times 10^{-31*}$	0.02	9.35×10^{-29}	1.33×10^{-30}	4.86×10^{-3}	0.01
Check(c)	1	388.39**	0.06	18.09**	0.88**	1.35**	0.05**	1.71
X (c)	37	34.52**	0.03	3.06**	1.91**	0.78**	0.07**	0.08
Error	3	0.04	0.03	0.01	0.02	0.10	0.01	0.01
Total	**43**							

Recommended cultural practices for the evaluation included fertilizer application, and fertilizers used included NPK (15-15-15) as basal application at a rate of 150 kg/ha during land preparation, and urea that was applied at the rate of 50 kg/ha as top-dressing first at tillering and a second time at booting. Morphological data were collected for 14 quantitative characters at appropriate growth stage of rice plant following the descriptor for rice (IRRI, 1980), making use of vernier caliper, meter rule, weighing scale. The characters that were evaluated are as shown in Table 2. The data collected on 14 agro-botanical traits from the rice accessions were subjected to statistical analysis using SAS/PC version 5 (SAS., 1982). Principal components grouping of the traits was employed to examine the percentage contribution of each trait to total genetic variation. Cluster analysis based on similarity matrices was also employed on agro-botanical data using the un-weighted pair group method with arithmetic mean (UPGMA) to obtain a dendrogram.

Random amplified polymorphic DNAs (RAPDs)

The analysis was performed on 40 rice populations. Total DNA was isolated from the leaves of 7 day old plants according to Dellaporta et al. (1983). Purified DNA was quantified by spectrophotometry and by ethidium bromide coloration after electrophoresis. Ten RAPD primers were used to generate markers as described by Tao et al. (1993). The amplification was performed in a reaction volume of 25 µl containing 10 mM Tris-HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mg/ml gelatin, Triton x100 0.1%, 0.1 mM of each dATP, dCTP, dGTP and dTTP (Promega), 10 ng of random primer, 50 ng of genomic DNA and 2 units of Taq polymerase. Amplification was carried out in a Mpi thermocycler as follows: 1 cycle of 3 min at 94℃; 44 cycles of 20 s at 94℃; 40 s at 37℃ and 1 min at 72℃; 1 cycle of 7 min at 72℃. Amplification products were then analysed for polymorphism after electrophoresis in 1.2% agarose gels in 0.5X TBE buffer and stained with ethidium bromide. For each primer, the consistent amplified products were recorded. Identified bands (loci) were sized and binary coded by 1 or 0 to indicate their presence or absence, respectively, in each rice accession. Genetic diversity (Nei, 1972) was computed from the binary data for all pair wise combinations of accessions. Genetic similarity among accessions based on observed RAPD marker data was also presented in form of a dendrogram using the UPGMA.

RESULTS

Table 3 presents the mean squares from analysis of variance for augmented randomized complete block design (RCBD) of fourteen traits measured in 40 rice accessions. Significant block effects were observed for flowering date, maturity day and plant height whereas block effects were non-significant for the other traits meaning that blocking was not important for the eleven traits that showed non-significant block effects. The two checks in each replicate as well as the experimentals differed significantly with respect to all 14 traits except leaf width and 100-grain weight.

Table 4 presents means of 14 characters as obtained for each of 40 rice accessions. Accessions 13 (CL SELECCION 3B) recorded the earliest flowering date (90 days) as well as earliest maturity date (120 days). However, accessions 28 (IR 45411-40-2-1) recorded the latest flowering and maturity dates of 117 and 147 days, respectively. Accessions 11 (CHANZI) that had the least number of total and filled tillers (10 in each case) was the tallest (155 cm) whereas TOX 3027-44-1-E4-2-2 that had the highest number of total and filled tillers recorded a plant height (113 cm) that was below the mean height (118 cm) of all 40 accessions. The longest panicle length of 30.3 cm was recorded for accession CHEIRO V whereas the shortest panicle length (21.8 cm) was recorded for IET 8565. Highest grain yield per plot was observed for accession IET 8113 with an average of 17 total filled tillers and medium maturity date of 118 days. However, IRRI accession IR 43450-SKN-506-2-2-1 with an average number of total and filled tillers of 16 and 15, respectively, also recorded the least grain yield.

Table 5 shows the correlation coefficients of pairs of 14 traits that were used in characterizing the 40 rice accessions. The correlation matrix showed that grain yield was positively and significantly associated with flowering date, maturity date, total tiller, filled tiller. Flowering and maturity dates were equally positively and significantly associated with total tiller and filled tiller. Also the latter two traits were positively and significantly correlated. Positive and significant association was also observed between grain length, grain width and 100-grain weight were both negatively associated with nearly all other traits.

The principal components analysis showing the factor scores of each character among 40 rice accessions, eigen values and percentage total variance accounted for by four principal components is presented in Table 6. The four principal components accounted for about 64.5% of total variance with the first principal component only taking 26.26%. The relative discriminating power of the principal axes as indicated by the eigen values was high (3.68) for axis 1 and low (1.46) for axis 4. The first principal component that accounted for the highest proportion (26.26%) of total variation was mostly correlated with total number of tillers, filled tiller, flowering and maturity dates and grain yield. Characters that were mostly correlated with the second principal component were plant height, tiller diameter, leaf length, grain length and grain width. The third principal component was dominated by traits such as grain length, 100-grain weight and to lesser extend by leaf width, plant height, flowering and maturity dates and panicle length. Only leaf and panicle lengths made substantial contribution to the fourth principal component.

Table 7 presents the characteristic means of the six similarity cluster groups in the 40 rice accessions. An examination of the range of means revealed that grain yield, plant height, total filled tiller, number of tillers and leaf length contributed the largest proportion of morphological variations that existed between cluster groups. There were no significant variations between clusters with respect to other traits.

The ten operon primers generated a total of 57 RAPD bands most of which were polymorphic across accessions because they were able to differentiate at least any two of the 40 rice accessions at a time. The number of amplification products per primer varied from one to ten with a mean of 5.7. Primers were able to produce fragments that varied from 200 to 5000 bp in size. As most of the RAPD primers were able to amplify more than one band per genotype, residual heterogeneity within lines is suspected.

Morphological dendrogram (Figure 1) shows the minimum distance between clusters and the extent of morphological relationship between pairs of accession within each cluster group. Accessions 18 and 21 in group 1; 25 and 39 in group 2; 4 and 33 in group 6, for instance were observed to be morphologically close in the dendrogram. Group 1 and 7 were morphologically different with more than 15 space units apart.

Table 4. Means of fourteen characters measured in 40 rice accessions.

DESIGNATION	FD (days)	MD (days)	PHT (cm)	TT	FT	TD (mm)	LL (cm)	LW (cm)	PL (cm)	PS %	GY (g)	GL (mm)	GW (mm)	GWT (g)
AFAAMWANZA 2-200	98	128	148	17	16	6.65	47.8	1.2	27.7	5	1786	9.2	3.2	2.4
ANDRAMONTA 3	102	132	136	13	13	5.43	40.3	1.3	25.0	5	1598	8.2	2.9	2.6
B4000J-KP-9-3	99	129	109	14	13	8.13	41.6	1.1	25.0	3	1709	8.7	3.1	2.2
B4354G-PN-75	99	129	118	13	12	5.85	43.1	1.3	25.3	1	1397	10.4	3.3	3.0
B5332-13D-MR-1-1	107	137	126	16	15	6.60	39.0	1.3	25.8	5	2688	9.3	3.2	2.8
BKN 6806-46-60	108	138	114	14	14	6.08	31.5	1.4	23.9	3	1536	10.6	2.9	3.2
BR 51-282-8-HR83	117	147	115	22	21	6.85	47.1	1.2	26.4	3	2774	9.4	2.8	2.6
BR 51-315-4	102	132	117	18	16	6.10	37.4	1.4	24.6	3	1186	9.5	2.7	2.2
BW 306-8	113	143	115	19	18	6.03	46.2	1.3	23.2	5	2314	6.7^{L}	3.2	2.0
BW 311-7	104	134	119	13	12	6.35	46.5	1.3	28.1	3	1944	9.2	3.1	2.8
CHANZI (BEIRA)	103	133	155	10^{L}	10^{L}	6.50	46.9	1.2	26.4	3	1162	10.9^{H}	3.0	3.0
CHEIRO V (NANIALO)	102	132	142	13	12	6.18	49.6	1.2	30.3^{H}	1	1430	9.4	3.2	2.8
CL SELECCION 3B	90^{L}	120^{L}	99	11	10	6.10	40.1	1.3	27.2	3	1546	9.1	2.3	2.2
CL SELECCION 450	93	123	105	11	11	5.98	44.9	1.6	25.1	1	1310	9.9	2.7	2.6
CN 836-3-10	114	144	124	17	16	6.28	50.2	1.7	26.9	5	1776	8.2	3.2	2.2
FACAGRO 59	105	135	125	21	20	6.63	46.8	1.4	26.6	3	3379	8.9	2.7	2.4
FAROX 318-1-3	108	138	108	19	19	6.18	45.9	1.5	28.7	3	3571	10.7	2.3	2.2
FOFIFA 47	95	125	114	18	18	5.25	47.5	1.5	25.7	5	1555	10.2	2.4	2.6
FOTSIFOTSY 156A	106	136	159	12	11	6.25	48.2	1.7	29.1	3	1421	8.9	2.8	2.2
IET 8113	112	142	118	18	17	5.75	46.1	1.6	24.6	3	3600^{H}	9.0	3.1	2.6
IET 8565	109	139	111	19	17	5.13	35.9	2.0	21.8^{L}	3	1541	7.9	2.9	2.2
IR 25077-KKN-38-PM-1-2-1	109	139	108	22	20	5.05	36.8	1.4	26.5	5	1752	9.7	2.6	2.4
IR 37096-50-1-3-3	111	141	119	19	18	5.50	33.5	1.2	25.2	5	2842	10.4	2.8	2.8
IR 41111-13-1-1-2	112	142	127	16	15	6.70	42.3	1.4	27.6	5	2218	10.5	2.9	2.8
IR 42221-2-2-3-1	101	131	96	14	13	6.05	29.5	1.2	24.5	3	1085	10.4	2.7	2.4
IR 42241-76-2-2-2	111	141	130	13	12	6.85	43.4	1.4	28.5	3	3096	9.7	2.7	2.2
IR 43450-SKN-506-2-2-1	102	132	124	16	15	5.13	33.7	1.2	27.5	5	1027^{L}	10.6	2.5	2.8
IR 45411-40-2-1	117^{H}	147^{H}	123	14	14	6.53	40.2	1.5	27.0	5	3014	10.5	2.7	3.0
IR 46375-CPA-19-3-1	111	141	133	14	13	6.33	48.1	1.3	26.7	1	1642	10.5	2.7	2.6
IR 48725-B-B-129-1	114	144	135	20	19	5.48	41.7	1.4	28.4	3	2938	9.3	2.6	2.4
ITA 222 (Check)	93	123	97	20	19	7.01	51.1	1.5	28.3	3	2663	9.1	2.9	3.1
ITA 324	100	130	103	12	11	6.83	52.2	1.3	29.6	5	1493	9.6	2.6	2.4
ITA 368 (Check)	104	134	102	16	15	6.83	48.4	1.6	26.2	5	1738	9.4	2.9	2.2
TCS 10	96	126	111	12	11	5.53	46.4	1.2	29.1	3	1550	9.6	2.8	2.6
TOX 3027-44-1-E4-2-2	111	141	113	23^{H}	22^{H}	5.65	44.4	1.5	25.8	3	2645	9.2	2.4	2.2
TOX 3052-46-3-3-2-1	112	142	122	20	19	5.35	46.8	1.4	23.6	3	3293	10.3	2.6	2.6
TOX 3081-36-2-3	102	132	102	21	20	5.98	40.4	1.5	26.8	3	2549	10.3	2.7	2.8
TOX 3093-4-5-3-3	111	141	111	14	13	5.15	45.9	1.4	25.3	1	3320	9.6	2.5	2.8
TOX 3098-4-2-1-2	105	135	97	19	17	5.73	33.1	1.6	26.1	1	2218	10.7	2.8	3.0

Table 4. contd.

TOX 3100-32-2-1-3	108	138	107	16	15	5.38	27.1	1.2	27.2	5	1978	10.2	2.8	2.4
Mean	105.4	135.4	118.33	16.64	15.69	6.19	43.63	1.40	26.63	3.4	2168.89	9.60	2.81	2.62
CV%	10.50	15.21	30.51	34.78	26.02	16.10	16.21	12.99	26.55	11.01	17.20	15.91	20.52	26.98
Lsd (5%)	2.52	3.51	4.00	2.57	1.82	0.04	0.31	0.81	0.31	1.00	166.11	1.22	0.59	0.31

NB:- H = Highest, L = Least.

Table 5. Correlation coefficients of 14 traits used in characterizing 40 rice accessions.

	GY	FD	MD	PHT	TT	FT	TD	LL	LW	PL	PS	GL	GW	GWT
GY	1.000	0.545**	0.540**	-0.071	0.514**	0.546**	0.026	0.166	0.146	0.002	-0.019	-0.022	-0.174	0.054
FD		1.000	0.998**	0.235	0.427**	0.408**	-0.111	-0.079	0.177	-0.172	0.153	-0.051	0.049	-0.077
MD			1.000	0.235	0.403**	0.408**	-0.110	-0.079	0.176	-0.172	0.152	-0.051	0.049	-0.078
PHT				1.000	-0.024	-0.022	0.076	0.287	-0.128	0.254	0.055	-0.062	0.318	0.037
TT					1.000	0.990**	-0.208	-0.080	0.248	-0.237	0.183	-0.137	-0.181	-0.154
FT						1.000	-0.205	-0.053	0.236	-0.237	0.209	-0.105	-0.205	-0.122
TD							1.000	0.376	-0.208	0.276	0.017	-0.108	0.350*	-0.043
LL								1.000	0.109	0.392	-0.013	-0.030	0.132	-0.099
LW									1.000	-0.024	-0.090	-0.022	-0.011	-0.020
PL										1.000	0.013	0.193	-0.115	0.045
PS											1.000	-0.192	0.037	-0.234
GL												1.000	-0.358*	0.560**

Table 6. Principal components analysis showing the contribution (factor scores) of each character among the 40 rice accessions, eigen values and percentage total variance accounted for by four principal components.

Character	Prin 1	Prin 2	Prin 3	Prin 4
Grain yield	0.36	0.11	0.21	0.34
Flowering date	0.40	0.23	0.26	-0.21
Maturity date	0.40	0.23	0.26	-0.21
Plant Height	-0.06	0.43	0.25	-0.22
Total Tiller	0.44	-0.09	-0.07	0.17
Filled Tiller	0.45	-0.09	-0.05	0.19
Tiller diameter	-0.15	0.37	0.02	0.21
Leaf length	-0.08	0.43	-0.06	0.47
Leaf width	0.20	-0.02	-0.29	0.08
Panicle length	-0.19	0.20	0.24	0.45
Panicle shattering	0.12	0.11	-0.17	-0.19
Grain length	-0.08	-0.35	0.54	0.09
Grain width	-0.09	0.38	0.00	-0.39
100-grain wt	-0.08	-0.17	0.52	-0.04
Eigen value	3.68	2.14	1.75	1.46
% variance	26.26	15.32	12.51	10.43
Cumulative % variance	26.26	41.58	54.09	64.51

Table 7. Characteristic means of six similarity cluster groups of 40 rice accessions.

Cluster group	1	2	3	4	5	6	Max	Min
Grain yield	3432.60	2249.60	2800.96	1114.80	1501.60	1811.66	3432.60	1501.60
Flowering date	109.60	110.00	109.22	102.00	100.92	105.14	110.00	102.00
Maturity date	139.60	140.00	139.22	132.00	130.92	135.14	140.00	130.92
Plant Height	116.80	112.58	117.58	123.00	120.29	116.68	123.00	112.58
Total Tiller	18.40	18.00	18.67	14.5	13.50	16.43	18.67	13.50
Filled Tiller	17.60	16.67	17.78	13.50	12.75	15.29	17.78	12.75
Tiller diameter	5.81	6.15	6.27	5.94	5.91	6.38	6.38	5.81
Leaf length	46.28	40.50	42.30	36.85	43.98	42.61	46.28	36.85
Leaf width	1.44	1.43	1.38	1.23	1.41	1.33	1.44	1.23
Panicle length	25.60	25.63	26.91	25.76	26.55	26.79	26.91	25.60
Panicle shattering	2.60	3.67	3.67	3.50	2.83	4.43	4.43	2.60
Grain length	9.7	9.30	9.69	10.35	9.53	9.23	10.35	9.23
Grain width	2.64	2.97	2.76	2.73	2.79	2.99	2.99	2.64
100-GWT	2.52	2.60	2.66	2.60	2.58	2.37	2.66	2.37

Table 8. Nucleotide sequence of selected primers with the number of amplified products and fragment size range (bp).

Primer	Sequence 5' to 3'	No. of polymorphic bands	Fragment size range (bp)
OPV10	GGACCTGCTG	8	300-2500
OPW03	GTCCGGAGTG	8	400-4200
OPW05	GGCGGATAAG	5	200-4800
OPW07	CTGGACGTCA	4	450-3800
OPW17	GTCCTCGGTT	4	900-3900
OPW19	CAAAGCGCTC	10	200-5000
OPS07	TCCGATGCTG	2	650-3400
OPT14	AATGCCGCAG	7	400-2900
OPT04	CACAGAGGGA	1	900-2000
OPR04	CCCGTAGCAC	8	750-3200

Interestingly, accessions 7 and 20 that out-yielded the check are in the same morphological group whereas accession 35 (Tox 3027-44-1-E4-2-2) with significantly more number of total and filled tillers was placed in group 7.

A plot of the first Principal Components Axis (Prin1) against the second PCA (Prin 2) (Figure 2) revealed that all the 40 rice accessions were ordered into four distinct PCA clusters. Also, all cluster groups of the morphological dendrogram with the exception of cluster 6, contributed one or more accessions to cluster 1 of the PCA, an indication that cluster 1 of the PCA contains a mixture of accessions mainly from clusters 3 and 7 that are morphologically similar. With the exception of 30, 33 and 34, all accessions in group 5 and 6 plus accessions 1, 6 and 8 of the morphological dendrogram were ordered into cluster 2 of the PCA. Thus, cluster 2 of the PCA is a conglomerate of accessions from all seven morphological dendrogram groupings. Most of the accessions in the third PCA cluster were also ordered from group 5 of the morphological dendrogram.

Figure 3 presents the molecular dendrogram of genetic similarity among 40 rice accessions as revealed by UPGMA cluster analysis based on RAPD markers. Pair of accessions 4 and 5; 7 and 8, 15 and 16 in group IX; 11 and 12 in group VIII; 22 and 24 in group VI, 38 and 40 in group V that had close to 90.0% similarity were accessions from the same background. For instance 4 and 5 are from Indonesia, 7 and 8 from Bangladesh, 11 and 12 from Mozambique. All the IRRI accessions used in the current study had between 60.0 and 90.0% genetic similarity and were thus clustered into four molecular

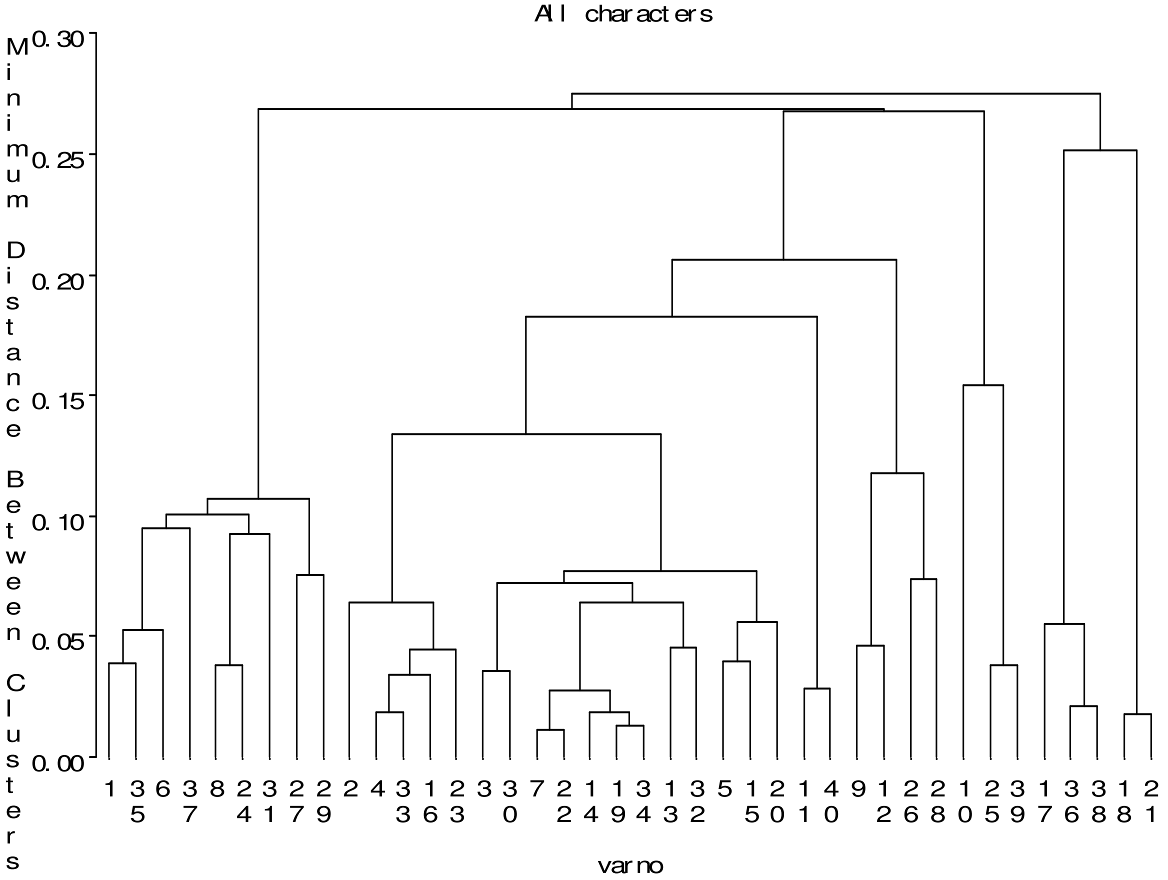

Figure 1. Morphological dendrogram showing the genetic similarity among 40 rice accessions.

groups. The two rice accessions from India IET 8113 and IET 8565 were genetically dissimilar and thus were far apart in the dendrogram. Accession TCS 10 from Mozambique had high 80% genetic similarity with accessions 35 and 36 from IITA. However, all other six IITA accessions including the check were generally similar. It was also observed that the IRRI accessions IR 45411-40-2-1 (28) that stood out in the dendrogram has some genetic similarity with TOX 3098-4-2-1-2 from IITA.

DISCUSSION

Variations did exist among the 40 rice accessions with respect to the 14 traits that were evaluated. Number of filled (fertile) tillers and total number of tillers on rice plants as well as heading and maturity dates were observed to greatly influence grain yield. On the other hand, leaf and panicle characteristics did not influence grain yield. Thus it was not surprising that positive and significant associations were observed between yield and days to flowering (r =0.545); days to maturity (r = 0.540); total tiller (r = 0.514) and total filled tiller (r = 0.546). However, panicle length was insignificantly associated with grain yield because, for instance, accessions CHEIRO and IET 8565 that had the longest (30.3 cm) and shortest (21.8 cm) panicle length respectively, recorded similarly grain yields.

The factor scores of the four characters: days to heading, days to maturity, total tillers and total filled tillers were mostly correlated with the first principal components of the Principal Components Axes. This observation, in addition to the characteristic mean values of the six similarity cluster groupings of accessions, confirmed the contributions of the four traits to grain yield among the 40 rice accessions. The implication is that if selection is to be made between cluster groups for a future breeding exercise, total number of tillers particularly filled tillers, days to heading, days to maturity and plant height should be given high priorities.

According to Aliyu et al. (2000) cluster analysis has the singular efficacy and ability to identify crop accessions with highest level of similarity using the dendrogram. Evaluation of phenetic diversity within rice accessions using the cluster and PCA analyses in this study has provided six and four clusters, respectively, with a lot of

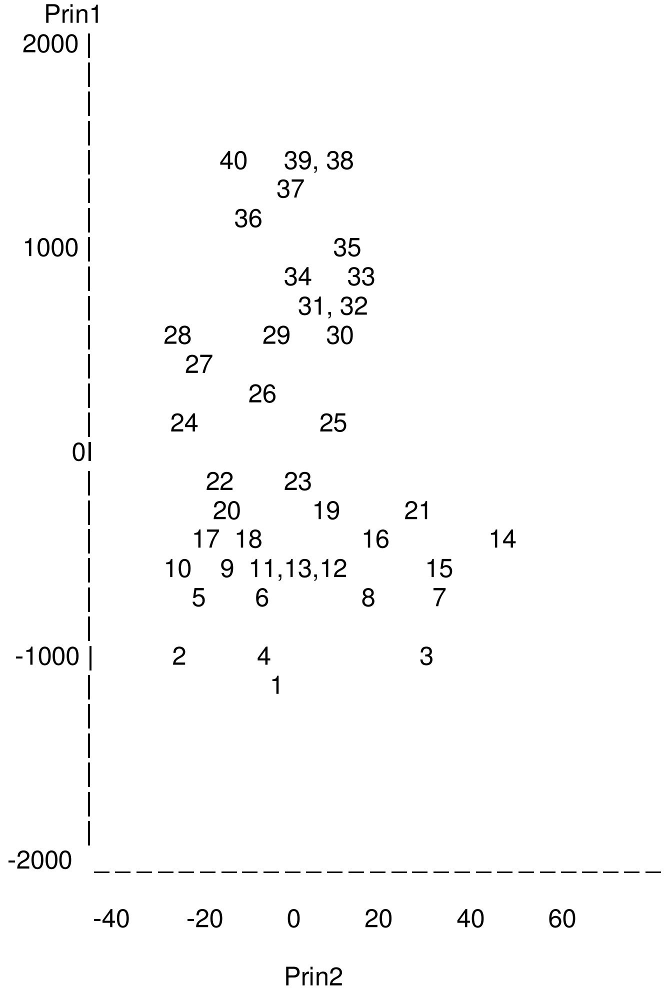

Figure 2. Plot of Prin1 and Prin2 showing the relationship between clusters of 40 rice accessions.

variations in morphological properties. Apart from the fact that the PCA was able to re-order accessions into four distinct clusters, the current study has shown that the use of morphological grouping could not provide convincing discriminatory evidence in the classification of rice accessions. It only provided a sort of minimum distance between groups of accessions.

Although, the morphological dendrogram generated from similarity or genetic distance matrices has provided an overall pattern of variation as well as the degree of relatedness among accessions, variations in environmental conditions such as soil types, and soil fertility levels (Steel, 1972); light, temperature and moisture regime (Summerfield and Huxley,1973; Morakinyo and Ajibade,1998), allow for different results to be obtained using morphological grouping, particularly when experiments are repeated in time and/or space. Also, both the genetic make-up of seed, environment and field management practices have been reported to influence the morphology of a crop (Singh and Rachie, 1985). According to Virk et al. (2000), the use of morphological characters in the classification of germplasm, particularly in rice, has been met with difficulties because the technique is inefficient. Observations above tend to emphasize the superiority of molecular similarity grouping over and above the morphological grouping. The eleven major clusters of the RAPD dendrogram together with their internal groups have demonstrated the polymorphic nature of the 40 rice accessions used in the current study.

The dendrogram obtained from the RAPD marker revealed that the marker was more discriminatory, highly polymorphic and thus, more informative than the one obtained from morphological characterization because the marker was able to make use of ten OPERON primers to generate 57 RAPD bands across the 40 rice accessions. Consequently, most of the bands were polymorphic as each band was able to differentiate at

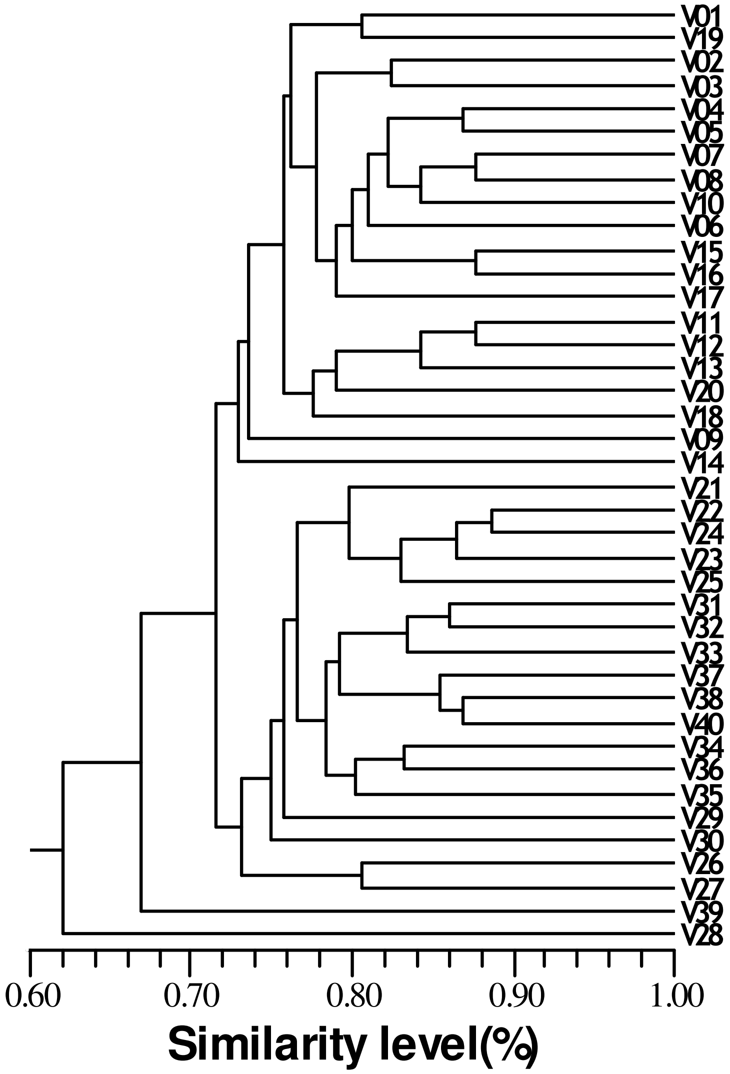

Figure 3. Molecular dendrogram showing the genetic similarity among 40 rice accessions revealed by UPGMA cluster analysis based on RAPDs.

least any two of the 40 rice genotypes. Thus, differentiation among rice genotypes was higher for RAPD markers than for morphological classification. The results are consistent with the morphological, allozyme and RFLP studies in sorghum (De Wet, 1978; Aldrich et al., 1992; Cui et al., 1995).

In conclusion, both morphological and genetic variations exist between the 40 rice accessions that were evaluated. Total number of tillers, number of fertile tillers, heading date, maturity date and grain yield contributed a greater proportion of variations that exist among cluster groups. The dendrogram obtained from the molecular was more discriminatory than the one obtained from morphological because the marker was able to make use of ten primers to sort out the 40 rice accessions into eleven cluster groups than the morphological which was seven. Principal Component Analysis revealed that all the 40 rice accessions were ordered into four distinct PCA clusters. By virtue of their highly promising performance in terms of yield, total tiller, total number filled tillers, cultivars IET 8113 from India, TOX 3027-44-1-E4-2-2 and TOX 3052-46-3-3-2-1 from IITA, and FACAGRO 59 from Burundi which performed better than checks could be selected with the Brazilian genotypes CL SELECCION 3B and 450 in a future rice breeding programs in Nigeria.

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