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Genetic Comparisons of Landrace Rice Accessions by Morphological and RAPDs Techniques

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Abstract: Morphological and molecular characterization of ninety-six landrace rice (*Oryza sativa* L.) accessions including six checks collected from four regions (North-West, North, West and Central-West) of Cote d'Ivoire were assessed using 14 agro-botanical traits and 10 Randomly Amplified DNA Polymorphisms (RAPDs) primers, respectively. Accessions were evaluated in a field experiment in an augmented experimental design with three replicates. The aim of the research was to study variations and to select lines that could be used as potential parents in future breeding programs. A principal components plot and a dendrogram based on distance between genotype cluster groups for mean values of the morphological variables were used to group the accessions. Genetic relatedness among accessions based on RAPD molecular marker was also presented in form of a dendrogram generated by clustering analysis using the Unweighted Pair Group Method with Arithmetic mean (UPGMA). The relative effectiveness of the RAPD markers compared to botanical descriptors in assessing diversity among the accessions was investigated. Clustering analysis technique using NTSYS classified the 96 landrace accessions into 8 morphological groups whereas PCA re-ordered the accessions into three broad groups that had within cluster similarities and inter-cluster differences in morphological variations. Reaction products (bands) of the RAPD analysis were highly polymorphic, more discriminatory and informative as they were able to differentiate more pairs of accessions than the botanical descriptors. Apart from checks, highest grain yield (2316 g/plot) was observed for accessions 46 (DNN 184) with an average of 12 filled tillers, plant height of 136 cm and medium maturity date of 136 days. It was observed that number of total and filled tillers *per se* was not a function of yield but rather, these traits were significantly associated with plant height and maturity date. Although, landrace rice accessions in Côte d'Ivoire is associated with relatively narrow genetic base, positive heterosis could be promoted if any of the Gagnoa (GGA) accessions from Central West of the country is used in a future hybridization program with Danane (DNN) accessions from west because of genetic distance between members of the two groups.

Key words: Characterization, cluster analysis, genetic diversity, landrace rice, *Oryza sativa* L., polymorphism RAPD, UPGMA

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops that provide food for about half of the world population, particularly in Asia, Africa and Latin America where the demand for rice is a top priority (Sasaki, 1999, 2002). Its adoption as a principal staple food is increasing in Africa whereas self sufficiency in rice production is declining as demand increases. 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 (Ogunbayo *et al*., 2005). The need for expansion of rice cultivation does not only depend on cultural practices and management, it also depends 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 landrace rice should form an important constituent of collection efforts because of their enormous in-built genetic diversity due

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to several generations of growing and selection by breeders and farmers. Landraces also constitute a good source of unique genes for stress tolerance, high yield stability, adaptability to the environments and genetic dynamics (Frankel *et al*., 1995; Guei and Traore, 2001). However, the utilization of these rice genetic resources had been limited to only adaptable genotypes (Caldo *et al*., 1996). Thus, 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). A number of landraces are still cultivated locally but most of them are being rapidly replaced by improved cultivars due to increasing narrowing of genetic base. The adoption of new varieties means that the area planted to landraces are gradually disappearing (Guei, 2000). Thus, reduced genetic variability underscores the need to collect landraces for *ex situ* conservation and to characterize them for future rice breeding programs at morphological and molecular levels because the evaluation of phenotypic diversity usually reveals important traits of interest to plant breeders (Singh, 1989).

Ogunbayo *et al*. (2005) carried out a phylogenetic evaluation of forty rice accessions using morphological and molecular techniques in which within cluster similarities and between cluster morphological differences were observed. Landrace rice differ from improved cultivars in adaptation to soil type, sowing and ripening periods and yield stability particularly, in regions where seasons are unpredictable (Frankel *et al*., 1995). However, they constitute a good source of unique genes for stress tolerance (Guei and Traore, 2001) and they are genetically dynamic. Despite these positive attributes, little efforts have been made to characterize and evaluate landrace rice accessions of West Africa origin. The objectives of this study were to investigate the morphological and molecular organizations of the existing diversity in ninety-six landrace rice accessions and to compare genetic relatedness among these accessions.

MATERIALS AND METHODS

Evaluation of morphological traits: The germplasm used in this study consisted of ninety-six landrace accessions including ten improved varieties in which six were checks. The materials were collected by the Genetic Resources Unit of the Africa Rice Center (WARDA) from four different localities Gagnoa, Danane, Touba and Boundiali and their respective surrounding villages, in the West-central, Western, North-Western and Northern parts of Côte d'Ivoire, West Africa (Fig. 1). The collections have since been maintained *ex situ* in the gene bank of Africa Rice Center (WARDA) at M'bé, Côte d'Ivoire. For the purpose of easy identification and retrieval, accession number comprising of three letters (to represent the site), plus a serial number was assigned to each collection. Thus, landrace collected from Danane bore the prefix DNN Gagnoa GGN while those from Touba and Boundiali bore the prefixes TBA and BDL, respectively.

The experimental study was conducted under irrigated lowland conditions during the year 2004 and 2005 wet seasons at International Institute of Tropical Agriculture (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 un-replicated entries for which there are insufficient seeds. Entries are then assigned to these plots in the form of an incomplete block design. The seeds were sown in the nursery bed before they were transplanted at 21 day old with a spacing of 20×20 cm. One seedling was transplanted per hill and the inter-plot spacing was 40 cm. A plot size of 1.2×5 m with 6 rows was used for each accession in the field.

Recommended cultural practices for the evaluation included fertilizer NPK (15-15-15) as basal application at a rate of 150 kg ha^{-1} during land preparation and urea was applied at the rate of 50 kg ha^{-1} as top-dressing first at tillering and a second time at booting. Morphological data were collected for fourteen quantitative characters at appropriate growth stage of rice plant following the descriptor for Rice *Oryza sativa* L. (IRRI, 1980). The characters that were evaluated are plant height, leaf length, leaf width, days to 50% heading, days to maturity, total No. of tillers at heading, No. of fertile tillers, panicle shattering, tiller diameters, panicle length, grain length, grain width, 100 grain weight and yield per plot. The data collected on 14 agro-botanical traits from the rice accessions were subjected to statistical analysis using SAS/PC version 9.1 (SAS, 1999) and NTSYS pc 2.0 (Rohlf, 1993). 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.

PHENOL REACTION

As mentioned by Oka (1958), the phenol reaction is a good criterion to discriminate between *Oryza sativa* sub-species, *Indica* and *Japonica* and it is equally effective with African rice (Kochko de, 1987). All the varieties were tested for their response to the phenol reaction and the grains were soaked in a 2% phenol solution for 48 h. The change in color of the hull was compared with that of grains soaked in distilled water (control) for the same period and comparison of grains, based on the change in color of the hull were indicated as (+) and (-) in Table 5.

RAPD analysis: Total DNA was isolated from the leaves of 7 day-old seedlings, grown in the green house, according to Dellaporta *et al.* (1983). Gene-based RAPD analysis was performed on ninety-six rice populations from the species *O. sativa* and *O. glaberrima.* 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). Each 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^{-1} 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°C; 44 cycles of 20 sec at 94°C; 40 sec at 37°C and 1 min at 72°C; 1 cycle of 7 mins at 72°C. Amplification products were then analysed for polymorphism after electrophoresis in 1.2% agarose gels in 0.5X TBE buffer and stained with ethidium bromide. Pairwise comparison of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic products was used to generate similarity coefficients using statistical software package NTSYS-pc 2.0 (Rohlf, 1993) and Jaccard coefficient of similarity (Jaccard, 1908). Genetic diversity (bp) was computed from the binary data for all pairwise combinations of accessions according to the Nei diversity index (Nei, 1972). The similarity coefficient was used to construct a dendrogram by the unweighted pair group method with arithmetic average (UPGMA) according to Sneath *et al.* (1973), Swofford *et al.* (1990) and Rohlf (1993).

RESULTS

Significant block effects were observed for leaf width, grain length and 100 grain weight 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 effect (Table 1). The six checks in each replicate as well as the accessions differed significantly with respect to all 14 traits.

Accessions 92 (CG 14) recorded the earliest flowering date (75 days), earliest maturity date (105 days) as well as longest panicle length (38.7 cm) (Table 2). CG 14 also had a yield of 1985 g per plot. However, accessions 68 (GGA 136) recorded the longest flowering date (113 days), longest maturity date (143 days), shorter panicle length of 32.2 cm and a yield of 1965 g per plot that was not statistically different from that of CG 14. Accession 93 (TBA 1) with shortest panicle length of 15.6 cm had a yield of 806 g per plot and 26 (BDL 85) had the least number of total tillers (5) whereas accession 20 had the highest (20). Also, accessions 10 and 11 had the least No. of filled tillers (4) whereas accessions 15, 20 recorded the highest No. of filled tillers, 19 in each case. Apart from the checks, highest grain yield per plot was observed for accession 46 (DNN 184) that had an average of 12 filled tillers, plant height of 136 cm and medium maturity date of 126 days. However, accession 10 (BDL 38-A) with an average No. of total and filled tillers of 6 and 4, respectively, recorded the least (429 g) grain yield per plot. Interestingly, accession 20 (TOG 5672) that had the highest No. of total (20) and filled (19) tillers recorded a yield of 760 g per plot probably because the entry was too tall (149 cm) and late maturing (130 days) compared with the highest yielder (DNN 184). It should also be noted that most of the GGA accessions had average plant height and thus, average yield per plot.

The correlation matrix showed that grain yield was positively and significantly associated with maturity date, total tiller, filled tiller, panicle length and grain length

Table 1: Mean squares from analysis of variance for augmented randomized complete block design for fourteen traits measured in ninety six rice accessions

Source	df	Yield	Grain width	Flowering day	Maturity day	Plant height	Leaf length	Leaf width	Total tiller	Filled tiller	Panicle shattering	Panicle length	Tiller diameter	Grain length	100 grain wt.
Block	2	50.89	0.00	1.17	0.72	1.17	0.00	0.04**	2.72	0.22	0.00	0.00	0.01	0.03*	0.00**
Check (C)	5	56.67×10^{5}**	0.74**	80.40**	159.23**	2056.88**	678.29**	19.98**	169.96**	147.75**	10.09**	8.50**	3.21**	1.55**	840.28**
Accessions	87	25.20×10^{4}**	1.96**	87.40**	145.09**	304.40**	588.73**	127.66**	11.33**	8.25**	15.38**	56.26**	17.20**	1.69**	138.71×10^{2}**
Error	10	30.49	0.09	0.57	0.66	0.97	0.09	0.66	0.92	0.76	0.66	0.09	0.66	0.66	0.66

*, ** Significant at 5 and 1% probability levels, respectively

Table 2: Means of fourteen characters measured in ninety-six rice accessions

Accession No.	Designation	Flowering DAS	Maturity DAS	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Total tiller	Filled tiller	Panicle shattering (%)	Panicle length (cm)	Tiller diameter (mm)	Grain length (mm)	Grain width (mm)	100 grain wt. (g)	Grain yield (g)
1	BDL100-B	107	137	116	55.4	2.3	7.0	7.0	1	25.7	4.4	9.1	2.0	2.74	615
2	BDL105	102	132	109	54.7	2.3	9.0	8.0	3	26.7	4.7	6.8	2.7	2.75	1104
3	BDL166	77	107	122	51.7	1.5	8.0	8.0	5	20.2	6.8	9.8	3.5	3.96	1535
4	BDL169	77	107	117	34.7	1.4	6.0	5.0	3	18.8	7.4	9.1	3.4	3.96	847
5	BDL18-A	97	127	140	51.8	2.1	7.0	6.0	5	30.7	4.7	8.7	3.4	3.08	496
6	BDL26	84	114	121	37.0	1.4	8.0	7.0	5	27.7	7.5	9.1	3.2	2.96	1081
7	BDL28-B	95	125	150	59.4	2.1	8.0	6.0	3	24.1	5.1	9.0	3.5	3.17	749
8	BDL29	85	115	126	38.3	1.4	12.0	9.0	3	22.1	6.5	9.9	3.4	4.13	1407
9	BDL30	85	115	139	52.2	1.8	8.0	8.0	5	20.5	7.3	8.5	3.4	3.72	1698
10	BDL38-A	81	111	125	37.0	1.3	6.0	4.0^{L}	3	29.1	7.2	9.0	3.0	2.85	429^{L}
11	BDL38-B	96	126	117	39.9	1.9	5.0^{L}	4.0^{L}	1	22.1	4.7	9.4	2.8	3.77	456
12	BDL4	93	123	125	39.3	1.7	9.0	8.0	1	24.4	8.3	8.1	3.4	2.95	1035
13	BDL44	88	118	135	42.6	1.6	11.0	9.0	3	18.1	7.6	9.6	3.5	3.83	1242
14	BDL45-A	81	111	139	44.2	1.4	9.0	8.0	3	21.1	7.0	9.2	3.1	3.28	1255
15	ITA 212 (Check)	102	132	103	42.7	1.9	20.0^{H}	19.0^{H}	3	24.9	7.3	8.9	2.7	2.98	2860
16	BDL46	83	113	116	47.0	1.4	9.0	8.0	3	19.4	6.5	8.0	3.3	3.79	1166
17	BDL50-A	88	118	148	67.9	1.7	6.0	6.0	5	25.5	8.1	7.5	3.4	2.83	997
18	BDL51	88	118	145	53.3	1.8	9.0	8.0	5	19.8	7.1	9.4	3.6	3.81	1368
19	BDL52	81	111	135	54.7	1.9	7.0	6.0	5	19.8	6.8	9.4	3.1	3.63	710
20	TOG 5672	100	130	149	51.5	2.0	20.0^{H}	19.0^{H}	7	36.4	6.1	7.5	3.0	2.85	760
21	BDL59	81	111	140	39.4	1.9	12.0	9.0	1	20.4	6.8	8.3	3.4	3.80	1179
22	BDL65	88	118	120	42.0	1.8	6.0	5.0	1	19.5	6.7	9.0	3.5	3.64	805
23	BDL72	86	116	119	56.7	1.8	8.0	7.0	1	17.7	7.3	8.8	3.6	3.57	1007
24	BDL76	77	107	121	45.6	1.4	9.0	6.0	5	17.9	6.7	9.5	3.0	3.64	1353
25	BDL84	77	107	103	68.2	1.4	9.0	7.0	3	17.7	7.2	8.9	2.7	2.56	1100
26	BDL85	97	127	125	54.2	2.0	5.0^{L}	5.0	3	23.4	6.7	8.2	3.3	3.43	784
27	BDL9	97	127	146	55.2	1.6	9.0	8.0	1	25.8	7.8	8.5	3.3	3.39	1489
28	BDL90	87	117	123	39.3	1.8	7.0	6.0	3	20.1	6.8	9.3	3.5	3.69	1137
29	BDL93	81	111	135	51.6	1.7	8.0	7.0	3	19.5	6.4	8.8	3.5	3.49	983
30	ITA 222 (Check)	102	132	100	34.8	1.9	20.0^{H}	18.0	5	21.5	6.8	8.8	2.9	2.91	3010
31	BDL97	84	114	104	34.0	1.6	8.0	7.0	1	19.2	6.5	8.5	3.6	3.48	950
32	DNN106	102	132	132	51.4	2.2	7.0	6.0	1	23.2	7.3	8.0	3.0	2.45	953
33	DNN111	102	132	131	52.4	2.2	9.0	8.0	1	25.9	7.8	8.4	3.1	2.93	1218
34	DNN116	101	131	138	57.6	2.3	12.0	11.0	3	25.3	7.0	9.6	2.5	2.73	1525
35	DNN117	95	125	142	57.2	1.8	7.0	7.0	1	25.1	6.7	8.7	2.3	2.50	914
36	DNN118	96	126	145	56.2	2.1	6.0	5.0	3	28.5	7.2	9.8	3.2	3.28	866
37	DNN12	96	126	141	54.5	2.1	9.0	7.0	3	27.8	6.4	9.0	3.0	3.45	1215
38	DNN123	97	127	141	60.9	2.1	8.0	8.0	3	26.4	7.3	9.2	3.0	3.27	1298
39	TOG 6202	89	119	107	49.1	2.1	14.0	12.0	7	35.4	6.0	8.7	2.7	2.48	780
40	DNN155	101	131	137	53.5	2.1	9.0	7.0	1	25.7	7.3	8.4	3.1	2.69	1127
41	DNN163	101	131	141	65.1	1.8	7.0	5.0	5	26.8	7.3	8.9	2.6	2.30	894
42	DNN180	96	126	138	58.6	2.2	8.0	7.0	5	27.1	8.1	8.4	2.8	3.09	931
43	DNN182	91	121	116	32.4	1.8	7.0	6.0	5	27.2	6.6	8.7	3.0	2.99	841
44	DNN18A	112	142	137	62.8	1.9	14.0	11.0	5	23.7	7.0	7.5	3.1	2.79	2115
45	ITA 306 (Check)	102	132	105	32.7	2.0	18.0	17.0	5	22.2	6.7	8.9	3.0	2.63	2740
46	DNN184	96	126	136	54.5	1.8	13.0	12.0	5	27.0	7.8	9.3	3.0	3.19	2316^{H}
47	DNN231	96	126	134	52.9	2.2	10.0	9.0	3	26.0	8.1	7.8	3.1	3.01	807
48	DNN232-C	102	132	156	41.8	2.1	13.0	12.0	1	26.5	6.5	8.5	3.0	2.67	1730
49	DNN234	97	127	138	55.1	2.1	8.0	7.0	3	30.5	7.3	10.0	2.9	3.09	1482
50	DNN239	96	126	140	48.7	2.2	8.0	7.0	3	30.9	7.6	9.4	3.0	3.07	1819
51	DNN240	96	126	132	58.9	2.2	12.0	11.0	3	24.7	8.3	9.5	3.1	2.97	1539
52	DNN244	96	126	139	52.8	2.4	12.0	10.0	3	26.4	8.6	9.2	3.0	3.08	1064
53	DNN249	96	126	133	49.1	2.3	12.0	11.0	3	28.0	8.3	9.4	3.0	2.94	1146
54	DNN257	96	126	154	67.3	2.3	13.0	12.0	3	28.1	8.3	9.3	3.3	3.07	2037
55	DNN270	97	127	131	53.5	2.1	8.0	7.0	1	26.7	5.7	9.6	2.9	2.91	955
56	DNN274A	112	142	133	40.2	1.7	15.0	14.0	3	26.1	7.3	8.0	3.0	2.65	2004
57	DNN274B	101	131	125	27.9	2.0	14.0	13.0	1	22.1	7.1	7.3	3.1	2.58	1978
58	DNN310	101	131	138	55.5	2.1	14.0	13.0	3	25.1	6.4	9.5	3.1	3.05	1341
59	DNN411	101	131	137	50.5	2.0	13.0	12.0	3	23.2	7.2	9.7	3.2	3.30	1884
60	NERICA 1 (Check)	101	131	103	40.9	2.0	10.0	9.0	1	21.9	7.1	9.6	2.8	3.12	2430
61	DNN546	101	131	140	55.2	2.2	10.0	9.0	3	28.4	7.5	9.5	2.8	2.94	933
62	GGA108	96	126	156	57.4	2.1	8.0	6.0	5	33.2	7.5	9.5	3.1	3.12	1156
63	GGA109	92	122	139	54.0	1.7	9.0	8.0	3	22.5	6.3	9.1	2.8	2.97	1256
64	GGA118	96	126	148	55.2	2.3	9.0	8.0	3	30.4	8.1	10.4	3.3	3.28	1545
65	GGA120	96	126	160	65.2	2.2	10.0	9.0	3	26.1	7.2	12.0^{H}	3.4	3.95	1338
66	GGA123	94	124	124	45.2	1.6	9.0	8.0	1	20.5	6.6	8.8	3.2	3.31	1515

Table 2: Continued

Accession No.	Designation	Flowering DAS	Maturity DAS	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Total tiller	Filled tiller	Panicle shattering (%)	Panicle length (cm)	Tiller diameter (mm)	Grain length (mm)	Grain width (mm)	100 grain wt. (g)	Grain yield (g)
67	GGA135	96	126	130	24.9	2.0	12.0	11.0	3	29.1	7.1	8.0	3.3	3.06	1186
68	GGA136	113[H]	143[H]	157	52.3	1.7	13.0	12.0	3	32.2	8.2	9.8	3.0	2.88	1965
69	GGA145	97	127	127	45.8	1.5	9.0	8.0	1	23.2	8.2	9.0	3.2	3.02	1315
70	GGA146	96	126	126	42.3	1.6	6.0	6.0	1	22.7	7.1	9.3	2.9	3.06	773
71	GGA151-A	96	126	149	64.5	2.5	8.0	7.0	5	27.5	6.2	10.3	2.9	3.21	1439
72	GGA151-B	96	126	156	57.0	2.5	8.0	7.0	1	26.2	6.4	8.8	3.0	2.64	966
73	GGA153	96	126	150	60.8	2.3	8.0	8.0	3	27.3	6.3	8.9	3.1	2.97	993
74	GGA155	97	127	135	42.5	1.5	10.0	9.0	1	25.0	7.4	8.4	2.8	2.70	937
75	NERICA 3 (Check)	94	124	108	32.8	1.6	14.0	13.0	1	21.7	6.3	9.8	2.8	2.93	2345
76	GGA158-A	96	126	164	63.6	2.1	13.0	12.0	3	26.9	6.3	9.0	2.8	2.74	1403
77	TOG 7448	96	126	115	48.2	2.0	8.0	6.0	7	36.5	6.2	8.5	2.9	2.25	745
78	GGA162	92	122	148	43.7	2.0	8.0	8.0	1	25.6	5.2	9.3	3.1	3.24	1203
79	GGA165-A	96	126	160	59.7	2.3	6.0	6.0	3	25.8	6.1	8.4	2.7	2.47	764
80	GGA166	96	126	162	55.9	2.3	7.0	7.0	3	27.0	7.8	8.4	2.9	2.62	820
81	GGA171	99	129	140	53.2	1.7	8.0	8.0	1	23.6	7.1	8.3	3.0	2.52	1035
82	GGA178-B	96	126	144	53.3	2.2	10.0	9.0	3	25.8	7.0	8.9	3.0	2.61	842
83	GGA203	96	126	134	33.2	2.1	9.0	8.0	3	26.2	7.2	9.4	3.6	3.40	1144
84	GGA22	96	126	152	48.1	1.6	8.0	8.0	5	26.5	7.3	9.7	3.0	2.93	718
85	GGA315	96	126	131	48.7	1.7	14.0	13.0	3	28.0	7.6	9.1	2.3	2.21	1001
86	GGA316	109	139	148	50.4	2.4	12.0	11.0	3	27.6	8.7	8.7	2.8	2.73	1806
87	GGA418	101	131	140	30.0	2.3	7.0	5.0	3	25.6	7.3	7.1	2.2	1.99	720
88	GGA440	97	127	123	48.9	2.1	8.0	6.0	5	28.8	7.3	10.0	2.7	2.82	1208
89	GGA457	98	128	123	62.7	1.5	13.0	10.0	1	24.6	7.1	9.8	2.7	3.53	1224
90	WAB 56-104 (Check)	97	127	96	48.6	2.1	15.0	14.0	3	25.3	7.3	9.9	2.7	2.65	2007
91	GGA543	97	127	105	40.9	1.8	13.0	10.0	7	27.6	5.4	6.6[L]	2.2	1.20	873
92	CG 14	75[L]	105[L]	127	49.8	2.2	18.0	17.0	7	38.7[H]	6.5	10.0	2.8	3.10	1985
93	TBA1	86	116	119	28.4	1.0	7.0	7.0	1	15.6[L]	7.4	9.4	3.0	3.46	806
94	TBA23	83	113	93	49.1	1.4	8.0	6.0	1	19.2	5.8	9.2	2.6	2.62	969
95	TBA24	92	122	141	44.1	1.8	13.0	11.0	1	24.4	7.8	9.3	3.2	3.04	1212
96	TBA28	86	116	170	53.6	1.7	10.0	8.0	1	25.4	8.6	8.6	2.9	2.84	1542
	Mean	95	123	126	51.4	4.3	10.3	9.5	4	23.4	7.8	8.8	3.2	3.04	1348
	LSD (5%)	0.20	1.06	0.91	0.45	0.01	0.09	0.10	0.50	1.05	0.51	0.90	0.11	0.08	52.50

H: High, L: Low

Table 3: Correlation coefficients of fourteen traits used in characterizing ninety-six rice accessions

Characters	GY	GW	FD	MD	PHT	LL	LW	TT	FT	PS	PL	TD	GL	100 grain wt.
Grain yield (GY)	1.000	-0.435**	0.080	0.367**	0.121	-0.339**	-0.476**	0.754**	0.606**	-0.357**	0.415**	-0.417**	0.418**	-0.268**
Grain width (GW)		1.000	0.022	-0.713**	-0.046	0.817**	0.951**	-0.504**	0.044	0.219*	-0.724**	0.936**	-0.234*	-0.085
Flowering date (FD)			1.000	0.544**	0.007	0.056	0.147	0.135	0.197*	0.170	-0.001	0.112	-0.282*	0.263**
Maturity date (MD)				1.000	-0.009	-0.597**	-0.609**	0.452**	0.073	0.070	0.482**	-0.587**	-0.116	0.354**
Plant Height (PHT)					1.000	0.232*	-0.151	0.005	0.050	-0.532**	0.438**	-0.099	0.525**	-0.583**
Leaf length (LL)						1.000	0.821**	-0.420**	0.097	0.029	-0.447**	0.832**	0.039	-0.312**
Leaf width (LW)							1.000	-0.479**	0.043	0.340**	-0.729**	0.963**	-0.350**	0.047
Total tiller (TT)								1.000	0.799	-0.123	0.505	-0.455	0.184	-0.077
Filled tiller (FT)									1.000	-0.099	0.168	0.077	0.155	-0.257
Panicle shattering (PS)										1.000	-0.405	0.325	-0.673	0.841
Panicle length (PL)											1.000	-0.714	0.473	-0.344
Tiller diameter (TD)												1.000	-0.294	0.027
Grain length (GL)													1.000	-0.681
100 grain wt.														1.000

*, **, Significant at 5% and 1% probability levels, respectively

(Table 3). However, grain yield had negative but significant association with grain width, leaf length, leaf width, panicle shattering, tiller diameter and 100 grain weight.

Grain width was positively and significantly correlated to leaf length, leaf width, panicle shattering and tiller diameter. While flowering day were also positively and significantly correlated to maturity days, filled tiller, grain weight and maturity days were positively and significantly correlated to total tiller, panicle length and grain weight.

The three principal components accounted for about 78.48% of total variance with the first principal component taking 40.33% (Table 4). The relative discriminating power of the principal axes as indicated by the eigen values was highest (5.65) for axis 1 and lowest (2.07) for axis 3.

The first principal component that accounted for the highest proportion (40.33%) of total variation was mostly

Table 4: Principal components analysis showing the contribution (factor scores) of each character among the ninety-six rice accessions, eigen values and percentage total variance accounted for by three principal components

Character	Prin 1	Prin 2	Prin 3
Grain yield	-0.28	0.10	0.35
Grain width	0.38	0.16	0.15
Flowering date	0.01	-0.18	0.36
Maturity date	-0.27	-0.30	0.12
Plant height	-0.10	0.36	-0.07
Leaf length	0.31	0.29	0.15
Leaf width	0.39	0.08	0.20
Total tiller	-0.29	-0.02	0.45
Filled tiller	-0.10	0.13	0.62
Panicle shattering	0.19	-0.41	0.11
Panicle length	-0.35	0.10	-0.05
Tiller diameter	0.38	0.10	0.21
Grain length	-0.19	0.40	-0.08
100-grain wt	0.09	-0.51	0.01
Eigen value	5.65	3.27	2.07
Variance (%)	40.33	23.37	14.78
Cumulative % variance	40.33	63.70	78.48

Table 5: Phenol reaction that characterized the ninety-six rice accessions into *Indica* (Lowland) and *japonica* (Upland) sub-species

S. No.	Designation	Reaction	S. No.	Designation	Reaction	S. No.	Designation	Reaction
1	BDL100-B	-	33	DNN111	-	65	GGA120	-
2	BDL105	-	34	DNN116	-	66	GGA123	-
3	BDL166	-	35	DNN117	-	67	GGA135	-
4	BDL169	-	36	DNN118	-	68	GGA136	-
5	BDL18-A	+	37	DNN12	-	69	GGA145	-
6	BDL26	-	38	DNN123	-	70	GGA146	-
7	BDL28-B	-	39	TOG 6202	+	71	GGA151-A	-
8	BDL29	-	40	DNN155	-	72	GGA151-B	-
9	BDL30	-	41	DNN163	+	73	GGA153	-
10	BDL38-A	-	42	DNN180	-	74	GGA155	-
11	BDL38-B	-	43	DNN182	-	75	NERICA 3 (Check)	-
12	BDL4	-	44	DNN18A	-	76	GGA158-A	-
13	BDL44	-	45	ITA 306 (Check)	+	77	TOG 7448	+
14	BDL45-A	-	46	DNN184	-	78	GGA162	-
15	ITA 212 (Check)	+	47	DNN231	-	79	GGA165-A	-
16	BDL46	-	48	DNN232-C	-	80	GGA166	-
17	BDL50-A	-	49	DNN234	-	81	GGA171	-
18	BDL51	-	50	DNN239	-	82	GGA178-B	-
19	BDL52	-	51	DNN240	-	83	GGA203	-
20	TOG 5672	+	52	DNN244	-	84	GGA22	-
21	BDL59	-	53	DNN249	-	85	GGA315	-
22	BDL65	-	54	DNN257	+	86	GGA316	-
23	BDL72	-	55	DNN270	-	87	GGA418	-
24	BDL76	-	56	DNN274A	+	88	GGA440	-
25	BDL84	-	57	DNN274B	-	89	GGA457	-
26	BDL85	-	58	DNN310	-	90	WAB 56-104 (Check)	-
27	BDL9	-	59	DNN411	-	91	GGA543	-
28	BDL90	-	60	NERICA 1 (Check)	-	92	CG 14	-
29	BDL93	-	61	DNN546	-	93	TBA1	-
30	ITA 222 (Check)	+	62	GGA108	-	94	TBA23	-
31	BDL97	-	63	GGA109	-	95	TBA24	-
32	DNN106	-	64	GGA118	-	96	TBA28	-

+ = *Indica* (Lowland), - = *Japonica* (Upland)

correlated with leaf width, tiller diameter, grain width, leaf length, panicle length, total tiller and grain yield. Characters that were mostly correlated with the second principal component were grain length, plant height, leaf length, 100-grain weight, maturity date and panicle shattering. The third principal component was dominated by traits such as filled tiller, total tiller, flowering date, grain yield and leaf width. Out of ninety-six accessions only ten were reacted positively to phenol and thus classified as *Indica* while other were *Japonica* (Table 5).

The ten operon primers generated a total of 108 RAPD bands all of which were polymorphic across accessions because they were able to differentiate at least any two of the ninety-six rice accessions at a time (Table 6). The number of bands per primer varied from eight to fourteen with an average of 10.8. Primers were able to produce fragments that varied from 250-3000 bp in size. Residual heterogeneity within lines is suspected because all the RAPD primers were able to amplify more than one band per genotype.

Table 6: 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)
OPT17	CCAACGTCGT	12	750-2500
OPM14	AGGGTCGTTC	12	1000-2000
OPJ19	GGACACCACT	8	500-2500
OPI14	TGACGGCGGT	8	250-2000
OPH20	GGGAGACATC	10	750-2700
OPI3	CAGAAGCCCA	13	250-3000
OPS2	CCTCTGACTG	9	500-2500
OPT3	TCCACTAATG	11	500-3000
OPT4	CACAGAGGGA	14	500-1750
OPS8	TTCAGGGTGG	11	750-2000

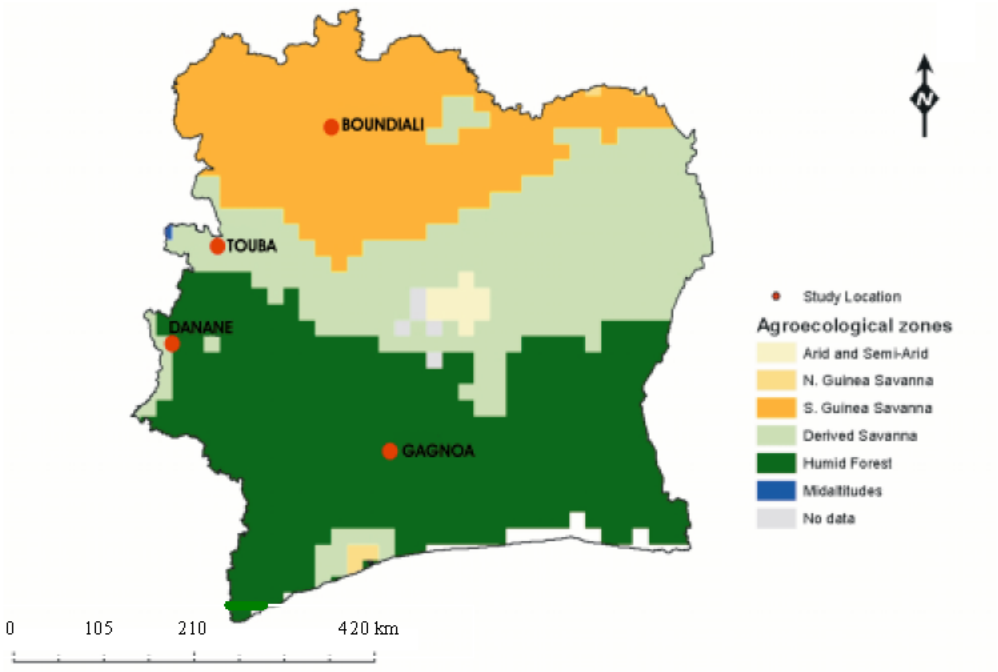

Fig. 1: Map of Côte d'Ivoire showing collection location

Accessions within clusters 1 and 7 were associated with about 20% similarity, those within clusters 5 and 6 had about 12.5% similarity whereas accessions within clusters 2, 3 and 4 were the most similar (about 6.3% similarity) (Fig. 2). Thus, to promote additional morphological variability among the 96 landraces, hybridization between accessions in clusters 3 and 4 (with highest distance) in one hand and those in clusters 1 and 7 (with least distance) on the other hand would be the most desirable. The dendrogram in Fig. 4 as opposed to that in Fig. 2 tends to suggest that most landrace rice accessions were morphologically similar with an average of about 12.5% morphological diversity.

A plot of relationship between the 96 landrace rice accessions as shown by the first and second principal components axes (Prin 1 and Prin 2) is shown in Fig. 3. Four out five accessions within cluster group plus accessions 74 and 93 of cluster group 4 were distributed at the top right side of the first principal axis. Accessions 8, 81 and 90 in cluster group 4 plus accession 83 in group 1 were also distributed towards the lower left side of first principal axis. However, all other accessions except 1,2,3 in group 7 were centrally distributed more towards the second principal axis and were thus, probably, morphologically similar. The above observations suggest within and between cluster variations resulting from large morphological diversity between the ninety-six accessions that were evaluated.

Figure 5 presents the molecular dendrogram of genetic similarity among ninety-six rice accessions as revealed by UPGMA cluster analysis based on RAPDs marker. The dendrogram shows a clear separation of the accessions into fourteen groups and allow discrimination between all 96 accessions compared with eight for the morphological grouping. A close phylogenetic proximity between TBA 1, TBA 28 and TBA 24 in group 9,

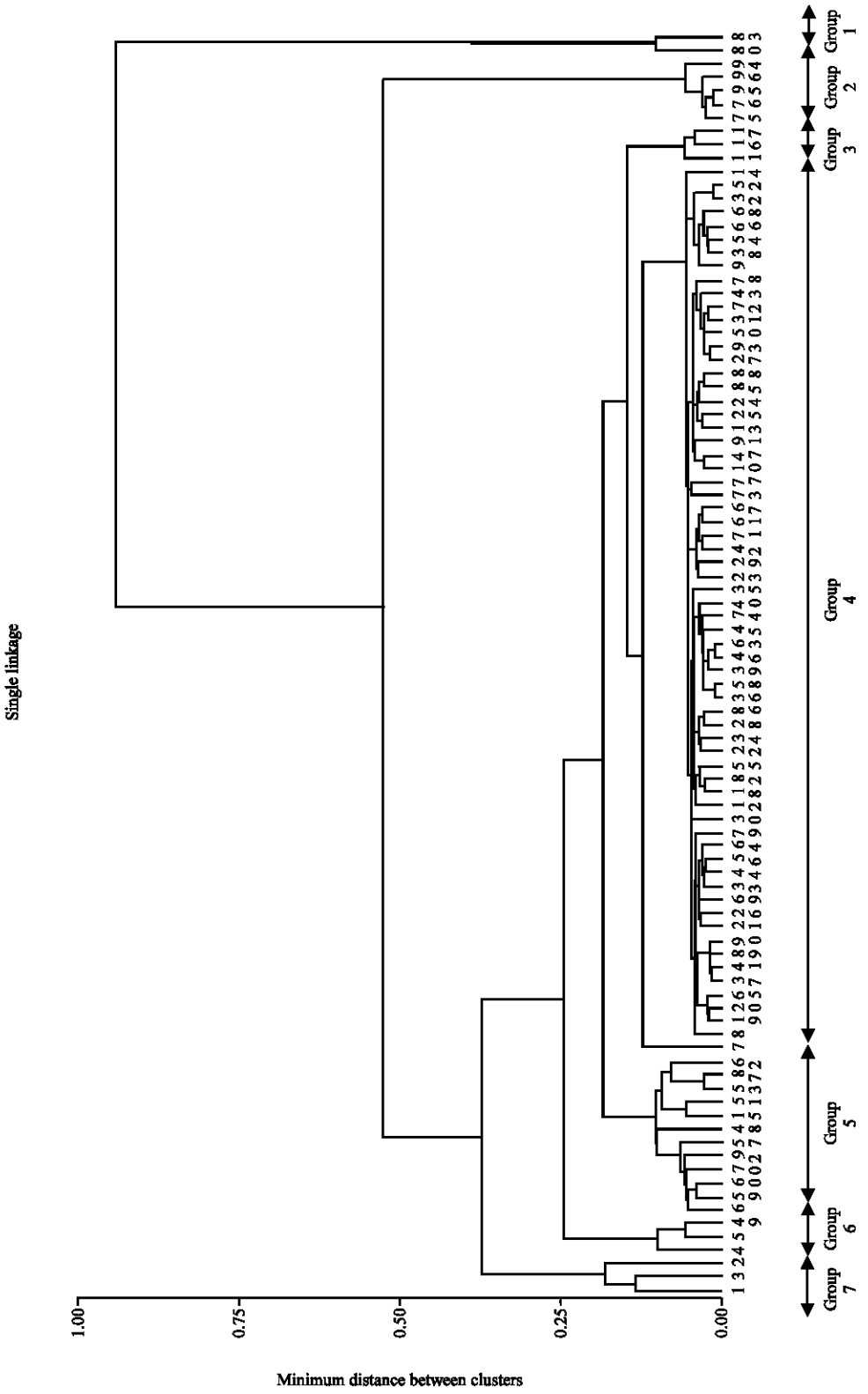

Fig. 2: UPGMA-based morphological dendogram showing the minimum distance between clusters groups

Fig. 3: Plot of prin 1 and 2 showing the relationship between clusters of ninety-six accessions (No. Represent accession numbers as in Table 3)

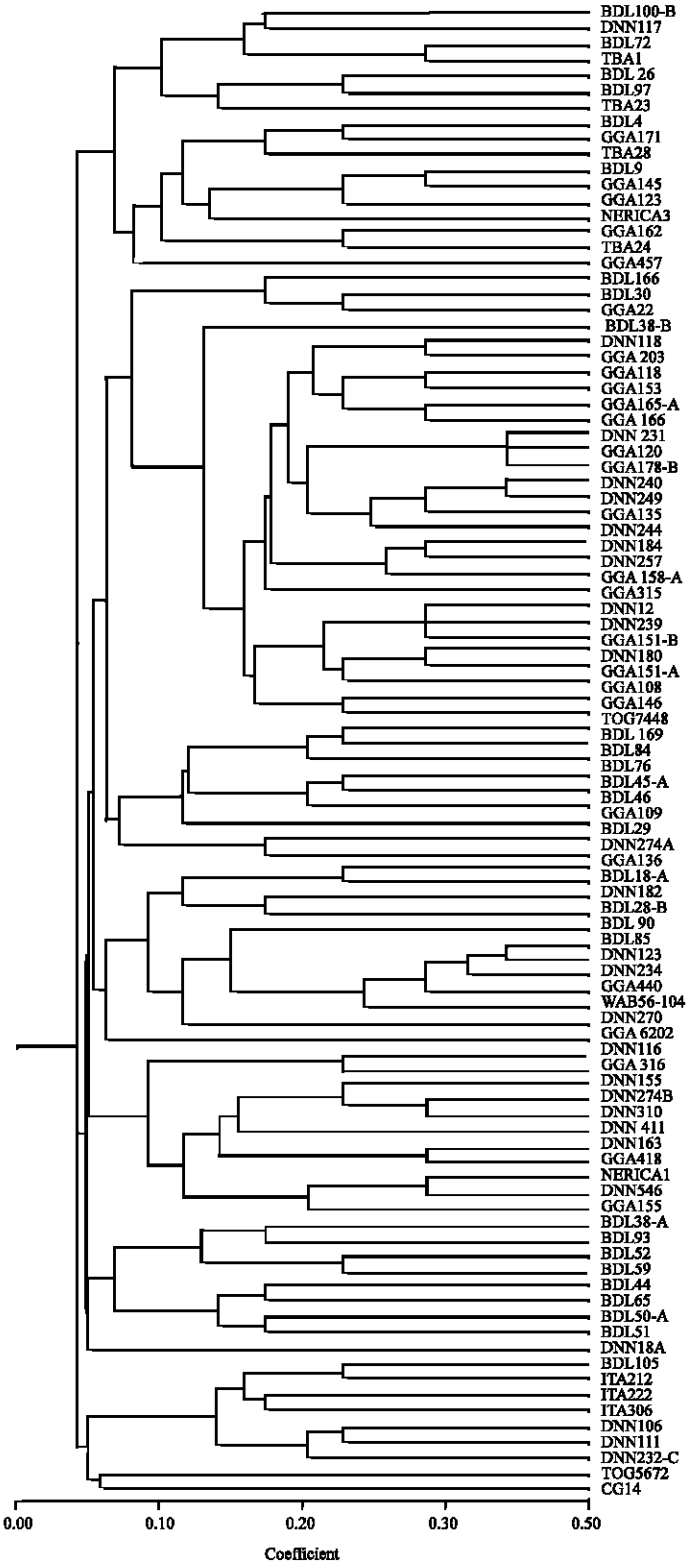

Fig. 4: UPGMA-based morphological using the NTSYS-pc 2.0 showing the genetic similarity among ninety-six rice accessions

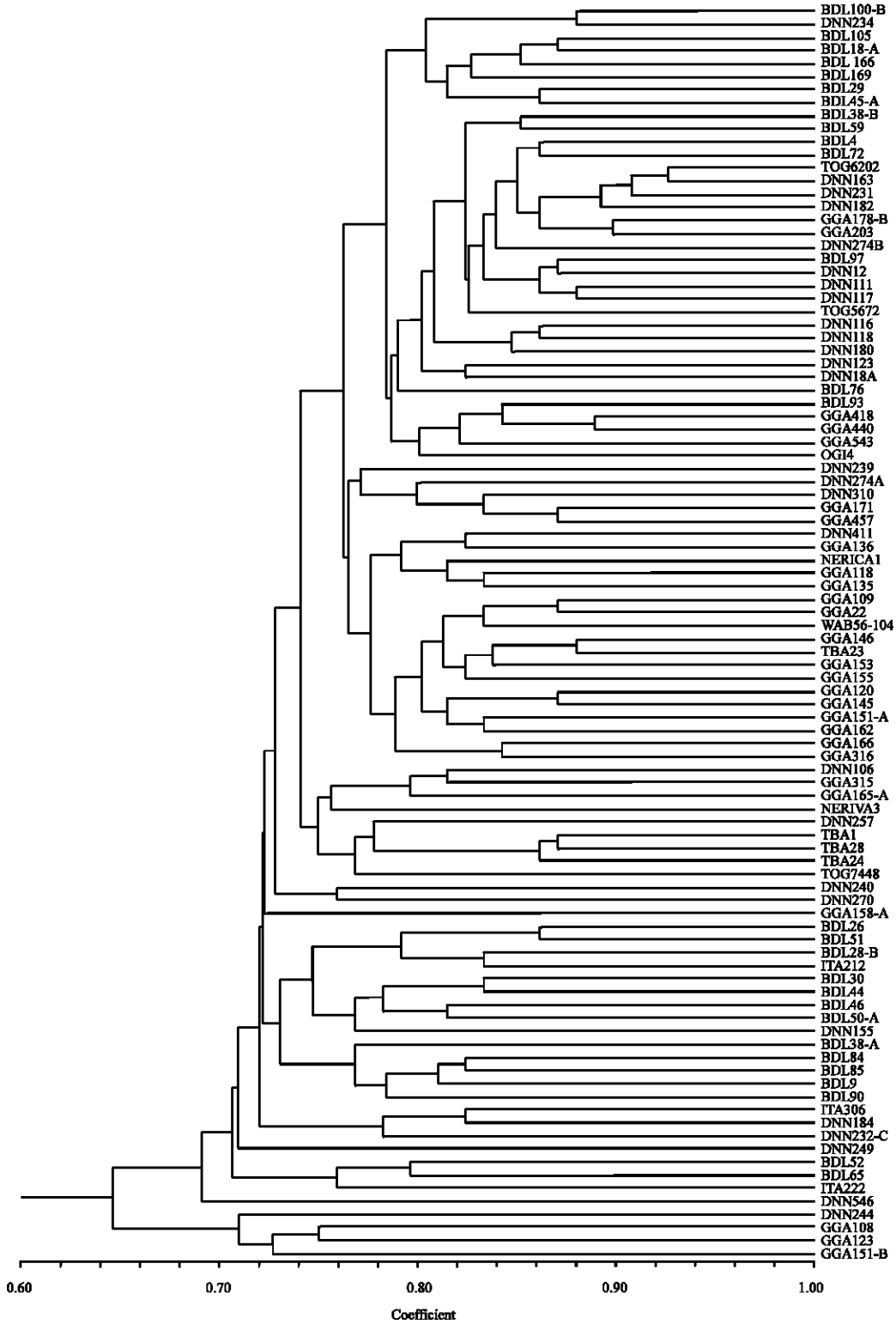

Fig. 5: Molecular dendrogram showing the genetic similarity among ninety-six rice accessions revealed by gene-based RPADs

accessions 20 (Tog 5672) and 39 (Tog 6202) in group 12 was shown. These results confirm previous data based on morphological markers (Table 2) that identified the five accessions as very similar in their vegetative apparatus and thus, very near from a taxonomical point of view. Pair of accessions 63 (GGA 109), 65 (GGA 120), 69 (GGA 145) and 84 (GGA 22) all in group 10 have very large (87.0%) similarity as they were from the same background. It was also observed that IITA accessions 15 (ITA 212), 30 (ITA 222) and 45 (ITA 306) were similar and had reasonable genetic similarity with accessions 7 (BDL28-B), 9 (BDL 30), 13 (BDL 44), 19 (BDL 52), 22 (BDL 65) and 46 (DNN 184).

Accessions 32 (DNN 106), 85 (GGA 315), 79 (GGA 165-A) and 75 (NERICA 3) in group 9 were genetically similar. Accessions 59 (DNN 411), 68 (GGA 136), 60 (NERICA 1) 64 (GGA 118) and 67 (GGA 135) in group 10 were equally genetically similar. However, the phenol test has indicated that all the above 9 accessions were japonica (upland) rice. Thus the RAPD marker has been able to confirm the phenol test. Heterosis can be promoted if any of the GGA accessions in group 1 can be used in a future hybridization program with accessions 39, 41, 47 in group 12 because of the genetic distance between members of the two groups.

DISCUSSION

Variations did exist among the ninety-six rice accessions with respect to the fourteen traits that were evaluated. Number of filled (fertile) tillers and total number of tillers on rice plants as well as maturity date were observed to greatly influence grain yield among the ninety-six rice accessions that were evaluated. On the other hand, flowering and plant height characteristics did not influence grain yield. Thus, it was not surprising that positive and significant associations were observed between yield and days to maturity (r = 0.367); total tiller (r = 0.754) and total filled tiller (r = 0.606). However, flowering date and plant height were insignificantly associated with grain yield because for instance accessions 68 (GGA 136) 92 (CG 14) that had the longest (113 cm) and shortest (75.0 cm) flowering dates recorded similarly grain yields.

The first principal component that accounted for the highest proportion (40.33%) of total variation was mostly correlated with leaf width, tiller diameter, grain width, leaf length, panicle length, total tiller and grain yield. Characters that were mostly correlated with the second principal component were grain length, plant height, leaf length, 100-grain weight, maturity date and panicle shattering. The third principal component was dominated by traits such as filled tiller, total tiller, flowering date, grain yield and leaf width.

According to Aliyu and Fawole (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 seven and three clusters, respectively, with a lot of variations in morphological properties. Apart from the fact that the PCA was able to re-order accessions into three distinct clusters, the current study has shown that the use of morphological grouping could not provide a convincing discriminatory evidence in the classification of rice accessions. It only provided a sort of minimum distance between groups of accessions. Also, 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.

However, due to 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), there is every tendency that different results are 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. Phenol reaction was able to classified the ninety-six rice accessions into sub-species group, *indica* (lowland) and *japonica* (upland). Out of ninety-six accessions only ten were reacted positively to phenol and thus classified as *indica*.

Observations above tend to emphasize the superiority of molecular similarity grouping over and above the morphological grouping. The fourteen major clusters of the RAPD dendrogram together with their internal groups have demonstrated the polymorphic nature of the ninety-six rice accessions used in the current study. The dendrogram obtained from the RAPD marker has 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 108 RAPD bands across the ninety-six rice accessions. Consequently, some of the bands were polymorphic as each band was able to differentiate at least any two of the ninety-six rice genotypes. Thus, differentiation among rice genotypes was higher using the RAPD markers because these DNA markers are proven as efficient in the identification of phenotypic markers that are linked to agronomically important traits. Thus, such traits can be introgressed during the development of near isogeneic lines. The results are consistent with the morphological, allozyme and RFLP studies in sorghum (Dewet *et al*., 1978; Aldrich *et al*., 1992; Cui *et al*., 1995).

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