



ASSESSMENT OF GENETIC DIVERSITY AMONG RICE GENOTYPES USING AGRO-MORPHOLOGICAL TRAITS IN ABAKALIKI, EBONYI STATE NIGERIA

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ABSTRACT

The research was carried out with 61 rice genotypes, that comprised landraces, inbred lines, released varieties adapted to area and introduced varieties to study the genetic diversity and their relationship based on agronomic and morphological traits. The field experiment was carried out during the cropping season of 2012 and 2013 at the Rice Research Field of the Biotechnology Research and Development Centre, Ebonyi State University, Abakaliki, Nigeria. The experiments were laid out in a Randomized Complete Block Design (RCBD) with three replications. The rice seedlings were transplanted to a plot of 2 m x 1 m with spacing of 20 cm x 20 cm at twenty-one (21) days after seeding. Data were collected from seventeen agro-morphological traits to study the diversity pattern among them. The traits were analysed using cluster analysis and principal component analysis (PCA). The results indicated a considerably high level of variations among the studied genotypes; the landraces produced mainly late heading and maturing, tallest plants and longpanicles, while the exotic genotypes were mostly early and intermediate heading and maturing, short and medium in height. High yielding genotypes were observed among both landraces and exotic genotypes. The result of the cluster analysis showed the existence of wide variation among the rice genotypes and were grouped into four clusters independently of their origin, based on the average performance of each of the genotypes used in the study. The local (landrace) genotypes were distributed in all the clusters, while the exotic including IRRI lines, Indonesian varieties, IWA (inbred) lines and released varieties were limited to two and three clusters respectively. The clustering pattern revealed highest level of genetic diversity among the landraces compared to the exotic varieties or inbred lines indicating that landraces are indeed good sources of variability and as such should not be allowed to go into extinction. Moreover, principal component analysis identified primary traits to be looked out for in rice breeding programmes to include leaf area and leaf area index, panicle length, plant height, days to flowering, days to maturity and paddy yield.

Keywords: *Agro-Morphological Traits, Rice Genotypes, Genetic Diversity, Assessment, and Numerical Taxonomy*

Introduction

Rice (*Oryza sativa* L.) is the domesticated tropical C3 grass that belongs to the family *Poaceae*, a staple food for one third of the world's population (Chakravarthi and Naravermi, 2006). It accounts for more than 70% of human calorie intake, rich in fibre, vitamins and minerals and low in cholesterol and sodium (American Rice Inc, 2011). This suggests that rice is a very good food for energy and health, especially for hypertensive individuals. In order to feed the growing world population, continued crop improvement and development of high yielding rice varieties in breeding programmes is critical. The success of any breeding programme, however, depends on the selection of parents for hybridization, which should be

divergent for the trait under consideration (Banumathy *et al.*, 2010).

In selecting suitable parents for plant breeding programmes, it is very important to obtain first-hand information on nature and degree of genetic divergence within germplasm (Banumathy *et al.*, 2010). Evaluation and quantification of genetic diversity is, therefore, an important aspect of plant breeding (Siva *et al.*, 2010), and unlocking the genetic diversity for agronomical important traits very necessary to provide pre-breeding information upon which selection will act upon (Ubi, 2012). Breeding programmes select genetically diverse parents to enable desirable combinations in segregating

generations. Genetic diversity among commercial cultivars is on the decrease, making it difficult to find new genes from cultivars for further improvement of grain yield and quality, and sufficient resistance to biotic or abiotic stress, even in rice. This provides motivation for scientists to explore wild and related species, landraces, plant introductions and breeding lines, etc, to identify genes to meet these demands (Wang *et al.*, 2000).

For example, landraces have built-in genetic variability due to several generations of cultivation and selection by farmers within an environment. They are adapted to the area with some resistance to major pests and diseases prevalent in such areas. They are therefore sources of genes needed in breeding programmes. Similarly, plant introductions also contribute to improving genetic gains in breeding populations as indicated by Caldo (1996), where core ancestral parents contribute important genes with several characters in a number of rice varieties selected by breeders. Conscious efforts are always made in breeding programs to introduce exotic species or varieties in order to increase genetic diversity of the breeding population. When this is done, pre-breeding activity involves characterization to understand the phenotypic diversity of the population. Such information would be useful in determining present trends in rice breeding as well as assessing alternatives for improving current rice cultivars.

Genetic diversity within and among populations could be studied using conventional methods based on morphological, agronomical and biochemical data using numerical taxonomic techniques (cluster analysis) or biometrical techniques (Mohammadi and Prasana, 2003; Jaradat *et al.*, 2004; Matus and Hayes, 2002; Ahmad *et al.*, 2008) or using molecular markers. The objective of this study was to evaluate the genetic diversity and relationship among 61 rice genotypes using numerical taxonomy techniques.

Materials and Methods

Genetic Material

Sixty-one rice genotypes consisting of released varieties, landraces, introductions from Indonesia and interspecific lines/segregating progenies (IRRI germplasm) obtained from Genetic Resources Unit of the Biotechnology Research and Development Centre, Ebonyi State University Abakaliki (Table 1) were assessed in this study.

Experimental Site

This study was carried out at the research farm of Biotechnology Research and Development Centre, Ebonyi State University, Abakaliki, Nigeria during the rainy seasons for two consecutive years of 2012 and 2013. The experiments were laid out in a Randomized Complete Block Design (RCBD) with three replications. The rice seedlings were transplanted to plot size of 2 m x 1 m with spacing of

20 cm x 20 cm at twenty-one (21) days after seeding. Recommended cultural practices were followed.

Data Collection and Data Analysis

The standard evaluation system (SES) from the Rice Reference manual (IRRI, 1996) was used for all traits measurement and was taken at maturity for all traits except when otherwise stated. Data were collected for plant vigour (measured at 20 days after transplanting), days to 50% heading, plant height (cm), tiller number, leaf length (cm) and leaf width (cm), leaf area and leaf area index, number of productive tillers or number of panicle, panicle length, maturity date, 100 seed weight (g), yield, culm colour, seed colour, grain colour, and seed type. Data collected were analysed by using the Generalised Linear Model of SAS (SAS, 2004) to verify the phenotypic variation in the traits measured. The differences between pairs of genotypes for the traits were calculated using Fisher's least significant difference at 5% probability level. The traits measured in this study were standardized to unit variance prior to calculation of the Euclidean distances and clustering using Statistical package for Social Sciences (SPSS, 2012). Standardizing the data matrix eliminates scale differences and ensures that all attributes are equally important to the analyses. Distance matrix between genotypes was constructed by means of Euclidean Distance Coefficients. Clustering of genotypes into similar groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances. Dendrogram was also constructed using Gowers' Similarity coefficient following Unweighted Pair Group Mean of Arithmetic Averages (UPGMA) clustering strategy of SPSS. Clusters were characterized based on average performance of genotypes included in different clusters. The mean, standard error, coefficient of variation were also calculated for the genotypes in each cluster. Ordination techniques using Principal Component Analysis (PCA) was used to confirm the result of cluster analysis. PCA was done using SAS (System Analysis Statistics) programme.

Results and Discussion

Agronomical Traits Analysis

There were variability in the 61 rice genotypes based on morphological and agronomic traits studied. The result indicated highly significant differences ($P < 0.001$) among genotypes for all the agronomic traits, except seed weight, indicating a high level of variation among the rice genotypes. There were also significant differences ($P < 0.001$) in the performance of the genotypes in the two years for number of tillers, number of panicles, paddy yield, seed weight and plant height ($P < 0.05$). Genotype by year interaction was very highly significant ($P < 0.001$) for all the agronomic traits studied in this experiment.

Cluster Analysis

The data generated was also analyzed using cluster analysis to identify relatively homogenous groups of genotypes based on the characters they share. Relationships among 61 rice genotypes revealed by UPGMA cluster analysis are presented in Fig 1. Results indicated divergence among the genotypes into different clusters irrespective of their origin. Four clusters were generated from the dendrogram at Euclidian distance of 5. The names of the genotypes making up each cluster are shown in Table 2. Cluster I was made up of twenty two genotypes including four landraces (Arubus, Ogbese, Jiargwula and Nwandende), four Indonesian varieties; Situ Patenggang, Danav (u) Gaong, Inpari 13 and Jattluhur, four IWA lines (IWA 4, IWA 6, IWA 7 and IWA 9) two released varieties (Nerica 34 and Upia 1), and eight IRRI lines. Cluster II on the other hand consist of 32 genotypes, also cutting across the four groups of genotypes studied. These include four landraces (Agreement, Mass, Ihenkiri, Room and palour), four Indonesian varieties (Mekongga, Limboto, Cihorang, and Inpari10) and four released varieties (Faro 42, Faro 44, Upia 2 and Upia 3). The remaining twenty genotypes were IRRI lines (Table 4). Similarly, Cluster III was made of one landrace (Lady's finger), one Indonesian variety (Way rarem), an IWA line (IWA 10) and two released varieties (Faro 57 and Faro 52), while Cluster IV was made up of one landrace genotype (Fadama 42) and one IWA line (IWA 8). In summary, there was no correspondence in geographical origin as shown by the clustering in the dendrogram.

The overall composition of the clusters was not in any form based on the origin or source of each genotype, rather it was based on the average performance of each of the genotypes used in this research. The cluster composition showed that some genotypes collected from the same geographic origin were distributed in different clusters, though most of the IRRI lines fell in the same cluster. On the other hand, genotypes from different locations were clustered together. This is similar to the result of Kuleung *et al.* (2006) who stated that varieties belonging to different countries of origin were grouped in the same cluster. This was attributed to germplasm exchange among breeding programmes globally, leading to the sharing of common gene over vast areas (Reif *et al.*, 2005b). Similar result was reported by Shanmugasundaram *et al.* (2000), Nayaket *et al.* (2004) and Zenget *et al.* (2004). The landraces were diverged across all the clusters showing that the landrace genotypes had highest level of genetic diversity for most of the traits studied. This result is in agreement with the study of Tang *et al.* (2002) which stated that landraces are important genetic resources for genetic improvement of crops because they provide "adaptability genes" for specific environmental conditions. It also agrees with the work of Steele *et al.* (2009) which reported that partial

introduction of modern rice varieties without much disturbance of the local landraces increase the rice genetic diversity.

The IRRI lines, Indonesian varieties, and released varieties were limited to only three clusters, respectively. Most of the IRRI and FARO lines were grouped together in the same clusters suggesting that most of them might have come from the same parent(s). This result is similar to the work of Maji and Fagade (2002), which reported that most of the rice varieties bred in Nigeria since 1986 have common parents, pointed out that 67 percent of the released varieties in Nigeria originated directly from IRRI materials. He also reported genetic uniformity within upland rice varieties in Nigeria. This result is also in agreement with Cuevas-Pe' rez *et al.* (1992) and Montalban *et al.* (1998) who reported that commercial varieties released for both systems had a narrow genetic base. Guimara' es (2002) evaluated the Brazilian rice varieties and arrived at the same conclusion. Mishra (2002) considered the breeding approaches used in India and the varieties released in the last 30 years, concluded that, 'the genetic base is narrowing and this is a matter of concern'. Evidence was added by Rai (2003) when analyzing 29 varieties released in the Indian Kerala State.

The data generated from field trial of the genotypes in each cluster and for all the agro-morphological traits studied were further subjected to analysis for means. This was done to characterize the genotypes in each cluster. Results (Table 3) indicated that Cluster I consist of late heading and maturing genotypes, tallest plants with long panicle, genotypes with highest number of tillers and panicles, and genotypes with highest values for leaf area and leaf area index. Conversely, Cluster II was made up of early heading and early maturing genotypes that produced shortest plants, but with very low values for leaf area and leaf area index. Cluster III was constituted by the high yielding genotypes that were moderate in height, intermediate in values for heading and maturity, leaf area and leaf index (Table 4). Furthermore, Cluster IV was characterized by most vigorous, early heading, early maturing, low tillering and low yielding.

The cluster content showed that none of them contained genotypes with all the desirable traits that could be directly selected and utilized, rather the minimum and maximum cluster mean values were distributed in relatively distant clusters. However, Clusters I, III and IV, recorded desirable mean value for maximum number of productive traits viz., plant height, long panicles and number of panicles, grain yield, and earliness to maturity. This result showed that selection of genotypes between these clusters will likely give desirable traits of interest. Bose and Pradhan (2005) reported a similar result, and studied a genetic diversity in deep water rice genotypes. This

underlies the fact that hybridization between genotypes of different clusters is necessary for the development of desirable genotypes. Based on the performance of the best genotypes within the clusters, they may be directly selected for purification and released to farmers or may be used as potential parents in hybridization programs.

Principal Component Analyses

In the 61 rice genotypes, four (4) principal components accounted for 76% of the total variation. This suggests a strong correlation among traits being examined. Few traits were identified as active variables, while others were kept as supplementary variables (Table 4). Traits separating the first principal component were: Leaf area (0.42), leaf area index (0.42), panicle length (0.37), plant height (0.36), days to 50% heading and maturity (0.32) and paddy yield (0.30). Along the second principal component; traits that contributed to the variability were tiller number (0.51) and crop vigor (0.45). In the third principal component, traits that affected the relationship of the 61 rice genotypes were number of panicles (0.40) and seed weight (0.28). Quantitative traits significantly affected the separation of the rice genotypes. However, principal component analysis of the rice genotypes revealed diverse grouping pattern which in general supported cluster analysis.

The contribution of each trait to total variation in the experimental population was studied. Among the traits, leaf area and leaf area index, panicle length, plant height, days to 50% heading, days to 50% maturity and paddy yield were identified as traits of primary importance have been grouped under PC1. This was followed by tiller number and crop vigour(PC2) and panicle number and seed weight(PC3). These characters should be given importance during hybridization and selection in the segregating populations that follows. Similar result were reported by Caldo *et al.* (1996) working with rice varieties in the Philippines. The result confirms the result of cluster analysis. Although principal component analysis organized accessions with more morphological similarities but clusters also included the accessions from different or far off sites. All data presented in this study allowed the identification of cultivars with diverse agronomical and morphological traits that could be used as possible parents for rice improvement program. The result also agrees with Lie *et al.* (2010).

Conclusion

The results generated in this study indicated the presence of considerably high level of variation among the 61 rice genotypes studied. The landrace genotypes exhibited highest level of genetic diversity compared to all others. This indicated that landraces are indeed good sources of variability which is important for any plant breeding program and as such

should not be allowed to go into extinction. Progress in plant breeding requires new sources of genes to meet needs that may not be foreseen. Breeders should therefore include more traditional varieties in their hybridization work to take advantage of these resources and the desirable traits found therein. To maintain genetic variability in modern cultivars, breeders must properly select parents to be involved in a cross. Additional variability must be sought, for important traits of choice in breeding programs. Evidence (unpublished result) indicated over reliance on modern (exotic) varieties by farmers. Although the modern released varieties can be very useful, sometimes higher yielding and disease tolerant, it is important that landraces are conserved. As indigenous crops, they have a long history in a region and are adapted to local conditions. Further work may also be required at the molecular level using DNA marker technology to ascertain the genome size and variability, and the relationship among the 61 genotypes used in this study to explain the clustering pattern observed in the study, irrespective of geographical divergence. This will further clarify the potential for compatible hybridization within or between the groups of genotypes.

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Table 1: List of rice varieties used for the experiments

S/n	Name/Description	Source
Released Varieties		
1	Upia 1	AGRA-EBSU
2	Upia 2	AGRA-EBSU
3	Upia 3	AGRA-EBSU
4	Faro 42	NCRI/WARDA
5	Faro 44	NCRI/WARDA
6	Faro 52	NCRI/WARDA
7	Faro 57	NCRI/WARDA
8	Nerica 34	NCRI/WARDA
IWA Lines		
9	IWA 4	AGRA-EBSU
10	IWA 6	AGRA-EBSU
11	IWA 7	AGRA-EBSU
12	IWA 8	AGRA-EBSU
13	IWA 9	AGRA-EBSU
14	IWA 10	AGRA-EBSU
IRRI Lines		
15	IR 75395 -2B- B-18-1-1-1-4-1-3-B-5	IRRI
16	IR 82574 -566-2-3	IRRI
17	IR 75395 - 2B - B -B-18-1-1-1-4-1-3-B-14	IRRI
18	IR 79599-38-2-3-3	IRRI
19	IR 81889-63-3-1-3-2	IRRI
20	IR 73008 -138-2-2-2	IRRI
21	IR 73417 -4-2-3-2	IRRI
22	IR 81303 -94-2-2-2	IRRI
23	IR 77186 -122-2-2-3	IRRI

S/n	Name/Description	Source
24	IR 81305-65-2-3-3	IRRI
25	IR 74371 -78-1-1	IRRI
26	IR 82033 -7-2-1-1	IRRI
27	IR 70213 -10-CPA-42-3-2	IRRI
28	IR 77500-12-2-3	IRRI
29	IR 06N 171	IRRI
30	IR 06N 187	IRRI
31	Perbombong	IRRI
32	PURPLE	IRRI
33	IR 06A 119	IRRI
34	IR 07A 108	IRRI
35	IR 07A 135	IRRI
36	IR 06N 191	IRRI
37	IR 06N 159	IRRI
38	IR 06M 102	IRRI
39	IR 06N 223	IRRI
40	IR 06N 139	IRRI
41	IR 06N 184	IRRI
42	IR 07A 144	IRRI
	Landraces	
43	Agreement	Farmers Seeds
44	Argwula	"
45	Arubus	"
46	Fadama 42	"
47	Ihenkiri	"
48	Lady's Finger	"
49	Mass	"
50	Nwandende	"
51	Ogbese	"
52	Room And Palour	"
	Plant Introductions (Indonesian varieties)	
53	Inapri 10	Indonesia
54	Inpari 13	Indonesia
55	Ciherang	Indonesia
56	Mekongga	Indonesia
57	Situ Patenggang	Indonesia
58	Jatiluhur	Indonesia
59	Way Rarem	Indonesia
60	Danav (U) Gaong	Indonesia
61	Limboto	Indonesia

Table 2: Clustering pattern of the 61 genotypes

Clusters	Number of genotypes	Names of genotypes
I	22	IR 79599-38-2-3-3, IWA 7, Danav (U) Gaong, Situ patenggang, Arubus, Ogbese, IR 75395 - 2B - B -B-18-1-1-1-4-1-3-B-14, PURPLE, IR 06A 119, IR 81889-63-3-1-3-2, IR 75395 -2B- B-18-1-1-1-4-1-3-B-5, IR 77500-12-2-3, Inpari 13, Jatiluhur, IWA 4, IWA 6, Nerica 34, IR 82033 -7-2-1-1, UPIA 1, IWA 9, Jiargwula, Nwandende,
II	32	Faro 44, IR 06N 187, IR 81303 -94-2-2-2, Upia 2, Mekongga, Agreement, Limboto, IR 07A 135, IR 82574 -566-2-3, IR 06N 223, IR 70213 -10-CPA-42-3-2, Mass, IR 07A 108, IR 06N 139, IR 74371 -78-1-1, IR 06N 184, Ciherang, IR 06M 102, Faro 42, Upia 3, Ihenkiri, IR 06N 191, IR 07A 144, Inpari 10, IR 81305-65-2-3-3, IR 77186 -122-2-2-3, IR 73417 -4-2-3-2, IR 06N 159, Room and palour, IR 73008 -138-2-2-2, Perubombong, IR 06N 171- 32.
III	5	Faro 52, IWA 10, Lady's finger, Way rarem, Faro 57.
IV	2	IWA 8, Fadama 42

Table 3: Cluster means of different characters in 61 rice genotypes

Traits	I	II	III	IV
Days to Maturity	96.6	90.3	94.5	90.7
Plant height	128.5	113.4	126.0	120.7
Leaf Area	45.9	40.1	42.5	43.0
Leaf Area index	1.2	1.0	1.1	1.1
Number of tillers	21.3	19.0	20.9	17.6
Number of Panicle	14.5	12.0	13.3	11.9
Panicle Length	34.1	31.4	33.2	27.6
Paddy yield(ton/ha)	1.93	1.58	2.08	1.27
Seed Weight	2.6	2.4	2.6	2.3
Culm colour	1.2	1.0	1.2	1.0
Seed Colour	1.5	1.4	1.4	2.0
Grain Colour	1.3	1.2	1.0	2.0
Seed Type	3.6	3.3	3.2	3.5

Table 4: Variation among rice cultivars accounted for first four principal components

Eigenvectors	Prin1	Prin2	Prin3	Prin4
Crop vigour	-0.16	0.45	-0.01	0.37
Days to 50 % flowering	0.32	0.23	-0.51	0.11
Days to 50 % maturity	0.32	0.20	-0.52	0.11
Plant height (cm)	0.36	-0.38	-0.17	-0.14
Tiller number	0.25	0.51	0.22	-0.24
Panicle number	0.07	0.29	0.40	-0.29
Panicle length	0.37	0.32	0.11	-0.22
Leaf area	0.42	-0.23	0.26	0.10
Leaf area index	0.42	-0.23	0.26	0.11
Grain yield	0.30	-0.01	0.09	0.22
Seed weight	0.07	0.08	0.28	0.75
Proportion	0.35	0.16	0.15	0.10
Cumulative proportion	0.35	0.51	0.67	0.76