

Original Research Article

Application of **multivariate** analysis to access selected rice germplasm phenotypic diversity

ABSTRACT

Without variability, it is not possible to conduct a plant breeding program. Germplasm is hence the critical first step in initiating a breeding program. Diversity in *O. glaberrima* accessions is enormous. It needs to be organized and characterized in order to facilitate its use by plant breeders. Two principal components (PRIN1) and (PRIN2) accounted for most of the variability observed in characters ~~measured~~ **studied**. PRIN 1 accounted for 56% of the phenotypic and morphological variation. The PRIN 1 was loaded on plant height, number of panicle, biomass wet weight, panicle wet weight and grain yield **traits**. PRIN 2 accounted for 23% of the variation. PRIN 2 was loaded on biomass wet weight, biomass dry weight, panicle wet weight, panicle dry weight, and harvest index **traits**. The test for univariate statistics described individual variable to explore pattern of response to variation showed strong statistical significance ($P < 0.001$) on phenotypic differences in all the variables that were measured. These traits **studied** are the most important ~~characters~~ contributing to the overall variability. The dendrogram produced grouping that defined **nine** distinct clusters and minimum genetic distance between clusters varies from 0 to **5.*****. All the selected *Oryza glaberrima* accessions and the *Oryza sativa* were distributed across the **nine** clusters respectively.

KEY WORDS: Principal Component Analysis, germplasm evaluation, genetic diversity, multivariate.

INTRODUCTION

Estimation of relatedness in crop species is one of the most appropriate way for parental selection and hybridization while selection of the parents is essential to enhance the potential yield increase (Islam, 2004). Cluster and PCA analysis have been used in the germplasm identification and parental selection, tracing the pathway to evolution of crop species diversification and origin (Bhatt, 1970; Carves et al., 1987; Eivazi et al., 2007; Mohammadi and Prasanna, 2003). In PCA analysis, the two first principal components in most studies contributed for high variation percentage will be a useful method to find the clusters (Fotokian et al., 2002). Many procedures have been used in studying of diversity in crop species, but cluster analysis have been used extensively for exploring both phenotypic and genetic diversity and grouping of plant materials with respect to the relationship of family based on their genetic material (Mohammadi and Prasanna, 2003). Mohammadi and Prasanna, (2003), reported that PCA and other techniques can be appropriately used for grouping crop genotypes into their distinct group while cluster analysis has been described an appropriate technique for determining relationships (Mellingers, 1972). A huge number of variable are regularly measured by plant breeders of which some may not be of sufficient

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39 discriminatory power for germplasm evaluation and characterization of traits (Shaibu and
40 Ogburia, 2002). In such a situation, principal component analysis (PCA) may offer a useful
41 insight in data set to eliminate redundancy. **Ulaganathan and Nirmlakumatri (2015) describe**
42 **that in** any crop, germplasm resource not only serves as a valuable source of useful genes
43 but also provides scope for building up a basic population of wide genetic variability.
44 Bringing improvement over existing crop varieties is a continuous process in plant
45 breeding. **However, To** achieve this objective, the breeder has to identify diverse parents
46 having high genetic variability for combining desirable characters. Therefore, knowledge of
47 sound genetic diversity is essential for undertaking any ~~recombination~~ breeding
48 program **targeting crop improvement. In earlier days, Ulaganathan and Nirmlakumatri (2015)**
49 **demonstrated in their study that** geographical diversity was considered as a measure of genetic
50 diversity but recently it is observed that genetic materials from same eco geographic origin
51 also possess diverse genetic makeup ~~and~~ **Thus**, it is not uncommon that the genetic materials
52 of different eco geographic origin possess similar genetic architecture ~~due to free and easy~~
53 ~~material transfer facilities~~. The usefulness of multivariate analysis for the study of
54 morphologically complex individual and ~~for measuring~~ the degree of divergence between
55 biological populations has been shown in different fields of research. Multivariate statistical
56 techniques ~~which simultaneously analyse multiple measurements on each individual under~~
57 ~~investigation are~~ **have been** widely used in analysis of genetic diversity irrespective of
58 whether it is morphological, biochemical or molecular marker-based and subsequently,
59 classification of germplasm collections. ~~Among the multivariate techniques~~ **It has been well**
60 **documented in literature that** principal component analysis (PCA) and cluster analysis had
61 been shown to be very useful in selecting genotypes for breeding program that meet the
62 objective of a plant breeder (Mohammadi and Prasanna, 2003). PCA has been reported to
63 reveal patterns and eliminate redundancy in data sets (Adams, 1995) while morphological
64 and physiological variations routinely occur in crop species. Cluster analysis is commonly
65 used to study genetic diversity and for forming principal subclass for grouping accessions
66 with similar characteristic into one homogenous category. Clustering is also used to
67 summarize information on relationships between objects by grouping similar units so that the
68 relationship may be easily understood for scientific knowledge and advancement.
69 Maji and Shaibu (2012) described that multivariate analysis has been used frequently for
70 genetic diversity analysis in many crops such as barley (*Hordeumvulgare*L.) **Sorghum**
71 **sorghum** (*Sorghumbicolor*L. Moench), wheat (*Triticum*spp.), peanut (*Arachishypogaea*L.)
72 (Upadhyaya *et al.*, 2009) ~~and~~ vineyard peach (*Prunuspersica*L. Batsch) ~~and in~~ rice (Ganaet

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al., 2013), and Multivariate analysis has been used frequently for genetic diversity analysis in many crops such as finger millet (Dagnachew et al., 2012b) and rice (Gana et al., 2013).

The main objective of any plant scientist is to identify an optimum number of plant characters which are sufficient to elucidate the maximum variability in field crop. This study was undertaken to run a classification analysis on selected *Oryzabambusa* accessions and Asian rice varieties by means of descriptive statistic and to understand the association of various characters, PCA and cluster analysis which would enable breeders to classify the available germplasm into distinct groups on the basis of their genetic diversity. PCA and cluster analysis allowed a natural grouping of genotypes (Bauer et al., 2007; Kraic et al., 2009). However, results work in wheat showed that cluster analysis based on PCA is a more precise indicator of differences among wheat genotypes (Reif et al., 2005; Fu et al., 2006; White et al., 2008). However, the results obtained would be valuable in developing an effective breeding.

MATERIALS AND METHODS

Plant materials, data collection and experimental design

The study was conducted to characterize 30 rice germplasm accessions consisted of 22 selected *Oryzabambusa* and 8 *Oryzasativa* lines obtained from AfricaRice gene bank were evaluated under lowland conditions at AfricaRice lowland breeding station, at the International Institute of Tropical Agriculture, Ibadan, Nigeria. IITA is located at (Latitude 7° 3' N and Longitude 3° 45' E in the forest-savannah agro ecology). These lines were evaluated in replicated trials in alpha lattice design during the dry season of 2014. The 21 day old seedlings were transplanted into a well levelled puddled field of 1.2m² plot at spacing of 20cm between rows and hills consisting two row plots of 3m length. Ten morphological traits were measured including days to flowering, plant height, number of tiller, number of panicle, biomass wet weight, biomass dry weight, panicle wet weight, panicle dry weight, harvest index and grain yield.

DATA ANALYSIS

Univariate statistics, standard deviation and probability level for each one of the 10 characters were calculated. Clustering of genotypes into similar groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances. For the ten agro-morphological characters, the data were standardized to have a mean of zero and a variance of one prior to squared Euclidean distance calculation.

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The principal component analysis method explained by Harman (1976) was followed in the extraction of the components and determined of the percentage variability explained by each component, so identify the patterns of morphological variation. The univariate descriptive statistics, cluster and principal component analysis were performed using the SAS version 9.2 software (SAS Institute, 1996, Cary, NC) of ten morphological traits in thirty rice genotypes.

RESULTS AND DISCUSSIONS

1- Univariate statistics:

The test for univariate statistics described individual variable to explore pattern of response to variation showed strong statistical significantly ($P < 0.001$) on phenotypic differences in all the variables that were measured (Table 1). This indicates that, the quantitative traits may be modified variously by the environmental conditions and each contributing such a small amount of phenotype that their individual effects cannot be detected by Mendelian methods but by only statistical methods. High variability observed for most of the traits indicated the possibility of improvement of these traits by direct selection. These traits should be given prominence in rice improvement.

Table 1: Univariate test statistics of some phenotypic characters identified by principal component analysis clusters.

Variable	Total standard deviation	Pooled standard deviation	Between standard deviation	R-Square	F Value	Pr > F
Days to flowering (days)	347.8	124.3	370.4	0.99	1743.2	<.0001
Plant height (cm)	14.1	11.9	8.5	0.32	10.1	<.0001
Number of tillers (#)	44.2	8.4	46.3	0.96	598.4	<.0001
Number of panicle (#)	121.9	28.9	126.4	0.94	373.0	<.0001
Biomass wet weight (gm^{-2})	114.8	91.6	76.5	0.39	13.7	<.0001
Biomass dry weight (gm^{-2})	188.8	139.0	139.6	0.48	19.8	<.0001
Panicle wet weight (gm^{-2})	60.89	52.8	34.4	0.28	8.35	<.0001
Panicle dry weight (gm^{-2})	57.3	54.4	22.9	0.14	3.5	<.0001
Harvest index (%)	63.3	35.9	56.0	0.69	47.6	<.0001
Grain yield (t ha^{-1})	195.3	79.2	191.1	0.84	114.1	<.0001

2- Cluster analysis:

130 Similarity indices and pattern of relationships among the *O. glaberrima* and *O. sativa* from
131 clusters and principal component analysis are useful in evaluating the potential breeding
132 value of both the checks and *O. glaberrima* lines. The checks - FARO 52, TOX 4004,
133 NERICA-L25, NERICA-U4, IR77298-14.1-2-B-10, IR74371-1-1, CG 14 and Apo were
134 distributed across six clusters. In Figure (1), the dendrogram produced grouping that defined

135 nine distinct clusters and minimum genetic distance between clusters varies from 0 to 5. The nine, first and second clusters consisted of one (TOG5396), two (TOG7442 and
136 TOG7996) and five (TOG6617, TOG6519, TOG13647, TOG12349 and TOG7400)
137 genotypes belongs to *Oryzaglaberrima*, respectively. The fifth cluster composed of three
138 genotypes belongs to *O. sativa* check i.e., CG14, IR77298 and IR74371. Similarly, the
139 seventh cluster comprised of three genotypes, but one genotype (FARO52) belong to *O.*
140 *sativa* check and two genotypes (TOMOYANG and RAM133) belongs to
141 *Oryzaglaberrima*. While, the third, fourth, sixth and eighth clusters composed of four
142 genotypes. The four genotypes belong to *Oryzaglaberrima* viz., TOG13429, TOG6335,
143 TOG6379 and TOG6408 in third cluster. During the fourth and sixth clusters, one genotype
144 belong to *O. sativa* check (NERICA-L25 and TOX4004, respectively) and three genotypes
145 belongs to *Oryzaglaberrima* i.e., (TOG6399, TOG9119 and TOG6410) and (A2-123,
146 TOG6311 and TOG6520), respectively. However, the NERICA-U4 and Apo genotypes
147 belongs to *O. sativa* check and the IRAT109 and TOG5534 genotypes belongs to
148 *Oryzaglaberrima* at the eighth cluster.

150
151 The results of cluster analysis suggested that there is genetic diversity among these genotypes
152 for studied traits. The standalone of one of the selected *O. glaberrima* accession may have
153 some underlying unique features not found in other groups (Figure 1). This work has
154 demonstrated the level of similarity between and within the selected lines. Hybridization
155 among these genotypes provided more possibility to having more phenotypic diversity and
156 could be used in breeding programs to achieve maximum heterosis and improvement of
157 studied traits in rice.

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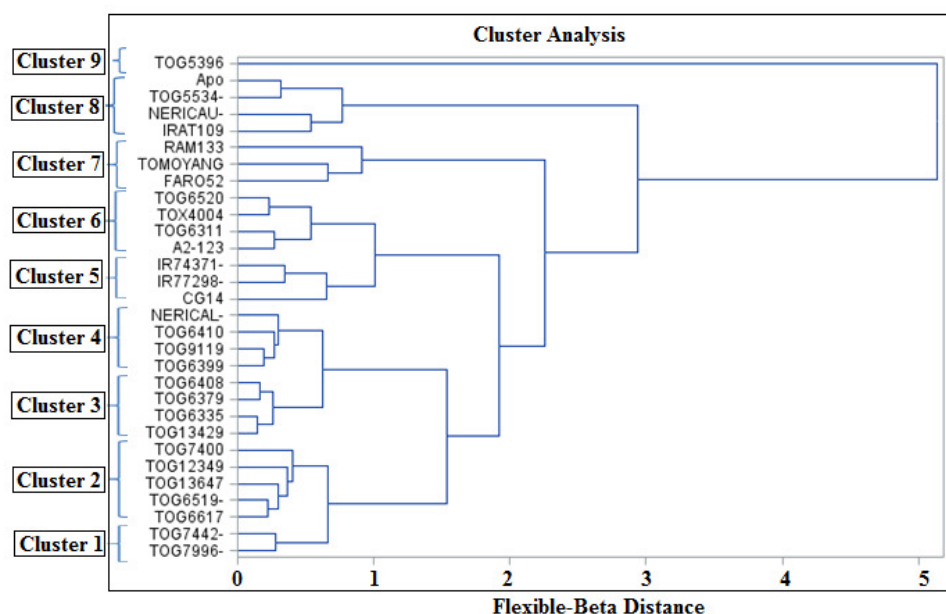


Figure 1. Clustering pattern and dendrogram of selected *O. glaberrima* and *O. sativalines*.

3- Principal component analysis:

The ten phenotypic traits measured, ten principal component axes were revealed from PCA analysis. Two principal components (PRIN1) and (PRIN2) accounted for most of the variability observed in traits measured (Table 2). The PRIN 1 accounted for 56% of the total phenotypic and morphological variation. The PRIN 1 was loaded on plant height, number of panicle, biomass wet weight, panicle wet weight and grain yield traits. The PRIN 2 accounted for 23% of the total variation. The PRIN 2 was loaded on biomass wet weight, biomass dry weight, panicle wet weight, panicle dry weight and harvest index traits. The most important descriptions were those associated with grain yield based on the analysis of natural variability in phenotypic traits measured in rice (Table 2). Days to flowering, number of tillers, biomass dry weight and harvest index traits negatively contributed in the first principal component axes (PRIN1), and only days to flowering negatively contributed to the total variation in the second principal component axes (PRIN2).

In general, we are interested in keeping only those principal components (PRIN1 and PRIN2) whose eigenvalues are greater than 1. The eigenvalues for PRIN1 and PRIN2 were 5.65 and 2.31, respectively. Components with an eigenvalue of less than 1 account for less variance than did the original variable (which had a variance of 1), and so are of little use. Hence, the point of principal components analysis is to redistribute the variance in the correlation matrix

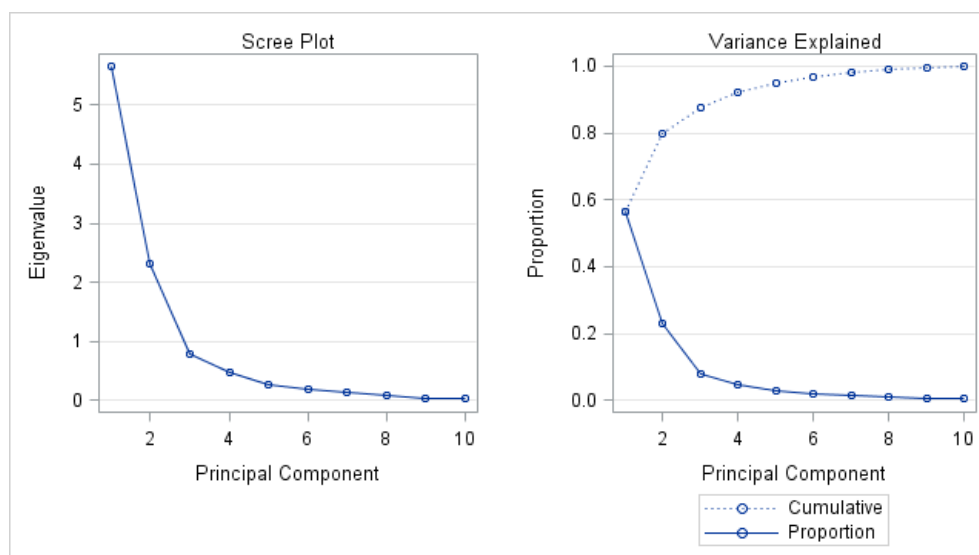
178 (using the method of eigenvalue decomposition) to redistribute the variance to first
 179 components extracted (Table 2). The scree plot graphs showed the eigenvalue against the
 180 number of component (Figure 2). The values in the two columns of the Table 2 showed two
 181 principal component axes. From the third component, it showed that the line is almost flat
 182 (Figure 1), meaning that each successive component is accounting for smaller and smaller
 183 amounts of the total variance.

184

185 Table 2: Eigenvector (“Weight”) and Eigen value (“Load”) of the correlation matrix and their
 186 contribution to total variation in rice.

Descriptor variables	PRIN 1	PRIN 2
Days to flowering (days)	-0.39	-0.01
Plant height (cm)	0.23	0.01
Number of tiller (#)	-0.41	0.04
Number of panicle (m ⁻²)	0.40	0.03
Biomass wet weight (gm ⁻²)	0.28	0.27
Biomass dry weight (gm ⁻²)	-0.28	0.43
Panicle wet weight (gm ⁻²)	0.18	0.53
Panicle dry weight (gm ⁻²)	0.01	0.60
harvest index (m ⁻²)	-0.35	0.30
Grain yield (gm ⁻²)	0.39	0.11
Eigen value	5.65	2.31
Proportion	0.56	0.23

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189 Figure 2: Scree plot and variance explained by the principal component analysis

190

191 The evaluation of germplasm frequently comprises measurement of phenotypic characters or
192 agronomic traits of interests, such as resistance to pests and diseases and tolerance to
193 physiological stresses that are influenced by environment which they grow. The data
194 measuring different characters are most or often required by plant breeders. According to Ng
195 and Padulosi (1992) and (Peters and Williams, 1984), breeders continually working and
196 looking for variation in germplasm for use in crop improvement. Therefore, continuous
197 evaluation of germplasm should be done to broaden the genetic base of the species and
198 identification of additional genes of interest or alternative source that control a particular
199 attribute or trait for use in crop improvement. As demonstrated in this study, genetic
200 variability in crop species should be exploited further to identify traits of interest so as to
201 develop new rice varieties with high stability to resist or tolerate adverse environments and
202 biotic conditions demonstrated by Gana, (2006). Awopetu and Gana (1997) studied the
203 genetic relationship of some rice varieties and observed that origin, habitat and breeding
204 background contributed to variation in the rice population. Therefore this study is conducted
205 to explain variation among the selected *Oryzaglaberrima* accessions *Oryzasativavarieties* and
206 identify traits that contribute to variability in this population and for their possible
207 exploitation in breeding programs. However, consistent effort is therefore required to
208 evaluate rice germplasm collection and breeding rice adaptable to harsh African environment
209 high grain yield.

210

211 CONCLUSION

212 Variation in plant phenotypic traits among rice accessions is a common phenomenon.
213 Without variability, it is not possible to conduct a plant breeding program. Germplasm is
214 hence the critical first step in initiating a breeding program. Diversity in *O.*
215 *glaberrima* accessions is enormous. It needs to be organized and characterized in order to
216 facilitate its use by plant breeders. There is need to understand how this tremendous variation
217 originates and the manner in which it is organized or classified. We therefore need to know
218 the sources to which breeders may go to find *O. glaberrima* germplasm with enormous
219 variation to initiate their breeding programs, and the rationale for selecting materials for
220 breeding and improving rice adaptable to African environment. Crop production occurs in a
221 dynamic environment with every growing season, numerous factors that influence their

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performance, while some factors are within control; many are not. The weather, genetics, soil environment conditions represent broad categories of factors that influence crop adaptation and their usefulness in crop improvement must be deal with on a continual basis. Moreover, introduction of statistical tools has bought significant change in identifying crop adaptable to certain regions while PCA and cluster analysis have also paves the wave to improve rice productivity on the basis on genetic background and pattern of relatedness.

In the context of identifying the pattern of relatedness, the hierarchical approach of cluster analysis and principal component analysis found to be quite suitable. Each cluster thus represents similarity. The following conclusion is drawn in the present study in the context of identifying the pattern relatedness, cluster analysis and principal component method can be applied as demonstrated in this study.

Thus, this study revealed that there is variation among the rice genotypes studied and the first two PCA axes explained and contributed to the majority of variation. However, results showed that cluster analysis based on PCA is a more precise indicator, and can be a useful tool for the selection of the most efficient genotype

Thus, as demonstrated in this study, members of this group have been reported suitable for breeding programs aimed at improving the yield of crop species (Nersting et al., 2006; Saleem et al., 2006; Figliuolo et al., 2007; Hysing et al., 2008; Mantegazza et al., 2008; Aghaee et al., 2010). Similar to the findings by Ali et al. (2008) who reported that cluster analysis can be useful for finding high yielding wheat. Considering the ten main components, the first two components explained 79% of total variations in data. PCA and cluster analysis allowed a natural grouping of the rice genotypes and appropriate use of techniques for genotypes grouping have been reported (Bauer et al., 2007; Kraic et al., 2009). However, results showed that cluster analysis based on PCA is a more precise indicator and can be a useful tool for the selection of the most efficient genotype

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