

Assessment of Genetic Diversity Among Intra- and Interspecific Lowland Rice using Morpho-agronomic Traits and SSR Markers

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Original article

Keywords: rice, morpho-agronomic data, principal component analysis, SSR markers, polymorphic information content, genetic diversity

Posted Date: October 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-816797/v1>

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Abstract

Rice is staple food in many countries of Africa and a major part of the diet in many others. However, Africa's demand for rice exceeds production with the deficit of 40% being imported. One way to improve Africa's rice production is through breeding high yielding varieties suitable for the different environment conditions. This study was conducted to assess the genetic variability and stability performance of 48 lowland rice genotypes including 37 interspecific (*Oryza glaberrima* × *Oryza sativa* ssp. *indica*) and 11 intraspecific (*O. sativa* ssp. *indica* × *O. sativa* ssp. *indica*) in 12 environments in Nigeria, Benin Republic and Togo using Additive Main Effect and Multiplicative Interaction (AMMI) and Genotype+ Genotype x Environment (GGE) biplot models. The combined analysis of variance revealed significant differences ($P < 0.01$) among the genotypes, environments, and genotypes x environment interaction. Both the AMMI and GGE models identified NERICA-L8 and NERICA-LI2 as the best genotypes for cultivation across environments. Ouedeme environments in Benin Republic were the most stable and ideal for rice cultivation, while Ibadan sites were the most unstable. TOG 5681 had the least yield and was the most unstable across seasons.

Genetic diversity was analyzed using 22 important morpho-agronomic traits and 50 simple sequence repeat (SSR) markers and the results were subjected to principal components analysis (PCA). The results revealed that the first eight PC axes (PC1–8) accounted for 75.13% of the total variation, while PC1–4 accounted for 50.39% of the total variation among rice genotypes. However, 10 of the 50 SSR markers were polymorphic and generated 49 alleles (average = 4.9 alleles per locus), suggesting moderate to substantial genetic diversity among the rice genotypes. The polymorphic information content (PIC) ranged from 0.24 to 0.65, with an average PIC value of 0.45. Two structured populations were observed which clustered into five heterotic groups and an outgroup, respectively. This suggests that heterosis could be exploited in the next hybridization program by crossing one of the genotypes in any SSR marker-defined cluster, with the rice accession TOG 5681 in cluster I. The results of this study suggest that morpho-agronomic traits should be used to compliment SSR data in rice diversity studies, especially if a few polymorphic SSR markers are to be used.

Background

Rice is the staple food of approximately 50% of the global population, particularly in Asia, Africa, and Latin America, and represents a major source of calories in the human diet in urban and rural regions (Lee *et al.*, 2013). The adoption of rice as a principal staple food crop is particularly increasing in Africa (Sie *et al.*, 2005; Rodenburg *et al.*, 2006; Ogunbayo *et al.*, 2007), where it is currently grown and consumed in approximately 39 countries, thus becoming a commodity of strategic significance in the continent. However, rice yield in Sub-Saharan Africa is low ($\sim 2.1 \text{ t ha}^{-1}$), and plateaued between 2012 and 2018 (USDA, 2018). Nonetheless, the rate of increase in domestic rice consumption (8% per annum) exceeds the domestic rice production growth rate (6% per annum) (WARDA, 2008). Because the demand continues to exceed supply, the region relies on imported rice (WARDA, 2004, 2006; Ogunbayo *et al.*, 2005). In 2006, Africa accounted for 32% of global imports, amounting to a record level of 9 million tons. This situation is worsened by the upward trends in international and domestic rice prices.

Rice belongs to the genus *Oryza*, classified under the tribe Oryzeae, sub-family Oryzoideae, and family Poaceae (Gramineae). The genus *Oryza* was named by Linnaeus in 1753. Vaughan (1994) recognized 22 species, whereas Brar and Khush (2003) recognized 23 species in the genus *Oryza*. Of these 23 species, 2 (*O. sativa* L. and *O. glaberrima* Steud.) are cultivated and harbor a diploid genome (AA, $2n = 24$), while the remaining 21 are wild species distributed throughout the tropics and subtropics, 10 different genomes (AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, HHKK) (Vaughan *et al.*, 2003; Brar and Khush, 2003). Tateoka (1963) revealed the basic groups of species within the genus *Oryza* and referred to these groups as species complexes (Table 1). To increase rice productivity in Africa, interspecific crosses between *Oryza sativa* and *Oryza glaberrima* were attempted, but these attempts failed because of high spikelet sterility in the progeny (Ndjiondjop *et al.*, 2008). However, the crop improvement unit at the Africa Rice Centre (AfricaRice) successfully developed improved upland and lowland rice varieties, now referred to as the New Rice for Africa

Table 1: The distribution and genomic classification of rice species throughout the world

<i>Oryza</i> species	Genome type	Chromosome Number	Geographical Distribution
<i>O. sativa</i> complex			
<i>O. sativa</i> L.	AA	24	Worldwide: originally South & Southeast Asia
<i>O. glaberrima</i> Steud.	AA	24	Tropical West Africa
<i>O. barthii</i> A. Chev. et Roehr	AA	24	West Africa
<i>O. glumaepatula</i> Steud.	AA	24	Tropical America
<i>O. longistaminata</i> A. Chev. et Roehr.	AA	24	Tropical Africa
<i>O. meridionalis</i> Ng.	AA	24	Tropical Australia
<i>O. nivara</i> Sharma et Shastry	AA	24	South & Southeast Asia
<i>O. rufipogon</i> Griff.	AA	24	South & Southeast Asia, South China
<i>O. officinalis</i> complex			
<i>O. punctata</i> Kotschy ex Steud.	BB, BBCC	24	East Africa
<i>O. malampuzhaensis</i> Krish. Et Chandr.	BBCC	48	Kerala & Tamil Nadu
<i>O. minuta</i> J.S.Pesl. ex C.B.Presl.	BBCC	48	Philippines, New Guinea
<i>O. eichingeri</i> A. Peter	CC	24	East Africa & Sri Lanka
<i>O. officinalis</i> Wall. ex Watt	CC	24	South & Southeast Asia
<i>O. rhizomatis</i> Vaughan	CC	24	Sri Lanka
<i>O. alta</i> Swallen	CCDD	48	Central & South America
<i>O. grandiglumis</i> (Doell) Prod.	CCDD	48	South America
<i>O. latifolia</i> Desv.	CCDD	48	Central & South America
<i>O. australiensis</i> Domin.	EE	24	Northern Australia
<i>O. schweinfurthiana</i> Prod.	BBCC	48	Tropical Africa
<i>O. granulata</i> complex			
<i>O. granulata</i> Nees et Arn. ex Watt	GG	24	South & Southeast Asia
<i>O. meyeriana</i> (Zoll. Et Mor. ex Steud.) Baill.	GG	24	Southeast Asia
<i>O. ridleyi</i> complex			
<i>O. longiglumis</i> Jansen	HHJJ	48	Indonesia, New Guinea
<i>O. ridleyi</i> Hook. f.	HHJJ	48	Southeast Asia
Unclassified			
<i>O. brachyantha</i> A. Chev. et Roehr	FF	24	West & Central Africa
<i>O. schlechteri</i> Pilger	HHKK	48	Indonesia, New Guinea

Source: Brar and Khush, 2003.

(NERICA), from interspecific crosses between *O. sativa* and *O. glaberrima* (upland NERICA) and between *O. glaberrima* and *O. sativa* (lowland NERICA). Several upland NERICA varieties were released for production in different parts of Africa (Ndjiondjop *et al.*, 2008). NERICA varieties inherited the primary branching trait of *O. glaberrima* and the forked branching trait of *O. sativa*, which increased the spikelet number per plant, thus enabling the double cropping of rice and its rotation with legumes, which enrich the soil by fixing nitrogen. The breeding and success of NERICA rice in Africa highlighted the tremendous potential of making favorable combinations of useful genes from two cultivated rice species (Rodenburg *et al.*, 2006), implying that improvements in productivity, profitability and sustainability of rice farming in Sub-Saharan Africa are feasible. However, the proportion of parental genomic contribution to the progeny, and the extent of genetic differences among these lowland sister lines remain unknown at the molecular level (Ndjiondjop *et al.*, 2008). Three recent advances, namely, the development of microsatellite or simple sequence repeat (SSR) markers, the availability of functional markers such as expressed sequence tags (ESTs), and the completion of the rice genome sequence (International Rice Genome Sequencing Project, 2005) have revolutionized the field of rice genomic research. SSR markers are considered ideal for genetic studies because they are co-dominant, multiallelic, and highly polymorphic (even in closely related individuals) and are highly abundant and uniformly distributed in plant genomes (Pan *et al.*, 2006; Pan, 2010).

SSR markers have been effectively utilized for achieving many objectives in rice research, such as genome mapping (Temnykh *et al.* 2000; McCouch *et al.* 2002) and determining the genetic relationship between several sub-species (Ni *et al.*, 2002). Morphological markers have also been used to assess genetic variation. Although morphological markers are less expensive than genetic markers, they are influenced by the environment and by genetic phenomena such

as epistasis. In addition, morphological markers may present an altered phenotype that interferes with the needs of the farmers. It is, therefore, difficult to identify true genetic similarity and dissimilarity (Egesi, 2001). Genetic diversity is essential for ensuring long-term selection gain in genetic improvement and for promoting the rational use of genetic resources (Ortiz *et al.*, 2008). Thus, proper genetic characterization and evaluation should include both morphological and molecular characterization. In this study, we examined the genetic diversity of 48 intra- and interspecific lowland rice hybrids across three countries of West Africa in six environments, based on important morpho-agronomic traits and SSR markers.

Materials And Methods

NERICA – New Rice for Africa

Research at AfricaRice has led to a major success in the development of improved upland and lowland rice varieties from interspecific crosses between *O. sativa* and *O. glaberrima* (upland NERICA) and between *O. glaberrima* and *O. sativa* (lowland NERICA). Both species harbor the AA genome with minor sub-genomic differences, which do not hinder normal chromosome pairing and gamete formation in the hybrids (Nayar, 1973) (Figure 1).

Experimental Sites

Field experiments were conducted in three different locations: the International Institute of Tropical Agriculture (IITA; Ibadan, Nigeria), AfricaRice (Ouédémé, Benin), and the field of an independent farmer in Kpalime (Togo). Ibadan (latitude: 7°30'N, longitude: 3°58'E, altitude: 210 m above sea level [masl]) experiences bimodal rainfall distribution mainly from mid-March to early-November, with a mid-season dry period from mid-July to mid-August. The average annual rainfall in Ibadan is 1,250mm, and the annual mean temperature is 27.2°C during the dry season and 25.6°C during the rainy season. Ouédémé (1°47'E, 6°48'N, 72 masl) is a village of the Dogbo community situated approximately 20km from Lokossa in the Kouffo district in southwest Benin, and shows bimodal rainfall, with an annual rainfall of 1,300mm. Togo has a tropical climate. In the south, the rainy season lasts from mid-April to June and from mid-September to October. The narrow coastal zone, which receives approximately 890 mm of rain annually, is the driest region. The region of Kpalimé located at 250masl has an average temperature of 25°C and rainfall of 1,500 from March until November. The mean annual temperature varies from 26°C along the coast and in the mountains to 28°C in the northern plateau. Daily minimum temperatures of approximately 20°C are recorded in the mountains in August. Daily maxima of approximately 38°C occur in the north in March and April at the end of the long dry season (Figure 2).

Morpho-agronomic Evaluation

A total of 48 lowland rice genotypes, including 37 interspecific (*O. glaberrima* × *O. sativa* ssp. *indica*) and 11 intraspecific (*O. sativa* ssp. *indica* × *O. sativa* ssp. *indica*), were evaluated in this study at the experimental sites described above. All genotypes were collected from the lowland breeding unit and AfricaRice Genebank, Cotonou, Benin. Table 2 lists the names and pedigrees of all 48 genotypes. A randomized complete block design (RBCD) with three replications was used. Seeds of irrigated lowland rice genotypes were sown in a nursery bed, and seedlings were grown for 21 days before transplanting, while valley bottom and fringe were sown directly at a spacing of 20cm × 20cm. One seedling was transplanted/planted per hill, and the inter-plot spacing was 40cm. A plot size of 1m × 5m with 5 rows was used for each variety in the field. Data were collected from the 0.6 × 4.6m middle rows leaving the extreme rows on each side as borders. Plants were fertilized with NPK (15-15-15) fertilizer applied as a basal application at a rate of 200kg/ha during land preparation, followed by urea, which was applied at a rate of 100kg/ha as a top-dressing, first at tillering and again at booting. The plots were hand-weeded regularly to minimize weed infestation. Bird damage was controlled using bird scarer.

Table 2. List and the pedigree of the genotypes used in the study.

Plot	Designation	Parents	Plot	Designation	Parents
1	BW 348-1	-	25	NERICA-L37	TOG5681/4*IR64
2	FARO 44 (SIPI 692033)	SIPI 661044/SIPI 651020	26	NERICA-L38	TOG5681/4*IR64
3	FARO 51 (CISADANE)	PELITAI 1//IR 789-98-2-3/IR 2157-3	27	NERICA-L39	TOG5681/4*IR64
4	IR75866-18-30-19-WAB1	BC3-IR 64 / TOG 5681	28	NERICA-L40	TOG5681/4*IR64
5	IR 75866-2-18-23-WAB1	BC3-IR 64 / TOG 5681	29	NERICA-L41	TOG5681/4*IR64
6	IR 75871-4-29-13-WAB1	BC3-IR 64 / TOG 5681	30	NERICA-L42	TOG5681/4*IR64
7	IR 75871-8-14-21-WAB1	BC3-IR 64 / TOG 5681	31	NERICA-L45	TOG5681/5*IR64
8	NERICA-L6	TOG5681/3*IR64	32	NERICA-L46	TOG5681/5*IR64
9	NERICA-L7	TOG5681/3*IR64	33	NERICA-L48	IR 64/TOG 5681//4*IR 64
10	NERICA-L8	TOG5681/3*IR64	34	NERICA-L49	TOG5681/3*IR64
11	NERICA-L9	TOG5681/3*IR64	35	NERICA-L50	IR 64/TOG 5681//4*IR 64
12	NERICA-L12	TOG5681/3*IR64	36	NERICA-L53	IR 64/TOG 5681//4*IR 64
13	NERICA-L14	TOG5681/3*IR64	37	NERICA-L54	IR 64/TOG 5681//4*IR 64
14	NERICA-L15	TOG5681/3*IR64	38	NERICA-L55	IR 64/TOG 5681//4*IR 64
15	NERICA-L17	TOG5681/3*IR64	39	NERICA-L56	IR 64/TOG 5681//4*IR 64
16	NERICA-L18	TOG5681/3*IR64	40	NERICA-L60	IR 64/TOG 5681//4*IR 64
17	NERICA-L19	TOG5681/3*IR64	41	SUAKOKO 8	SIAM 25 / 3*MALUNJA
18	NERICA-L20	TOG5681/3*IR64	42	TOX 4004-43-1-2-1	BOUAKE 189 / ITA 222
19	NERICA-L26	TOG5681/4*IR64	43	WITA 7	TOX891-212-1-201-1 105/TOX 3056-5-1/TOX 3440-171-1-1-1
20	NERICA-L28	TOG5681/4*IR64	44	TOG 5681 (Parent)	-
21	NERICA-L32	TOG5681/4*IR64	45	IR 64 (Parent)	IR 5657-33-2-1 / IR 2061-465-1-5-5
22	NERICA-L33	TOG5681/4*IR64	46	WITA 4 (Check)	11975 / IR 13146-45-2-3
23	NERICA-L34	TOG5681/4*IR64	47	FKR 19 (Check)	MASHURI / IET 1444
24	NERICA-L36	TOG5681/4*IR64	48	FKR 54 (Check)	-

Morphological data were collected on 22 quantitative and qualitative traits at appropriate growth stages of rice plants, according to the Standard Evaluation System (IRRI, 2002) (Table 2). Variability among varieties was estimated using the FASTCLUS clustering procedure. Principal component analysis (PCA) was carried out, and components with Eigen values greater than 1.0 were considered. The existence of moderate phenotypic variation among the 48 rice genotypes was further validated using biplot analysis.

Genotyping Using SSR Markers

DNA was extracted from the leaves of 7-day-old seedlings of all 48 rice genotypes planted in a screen-house at the International Rice Research Institute (IRRI), Los Banos, Philippines. DNA was analyzed according to the protocol of Dellaporta *et al.* (1983). The quantity and quality of DNA were determined using a spectrophotometer. Fifty SSR primers pairs were used to genotype 48 rice varieties by PCR, which was conducted in a 10- μ l reaction volume containing 1.0 μ l of 10X buffer, 2 μ l of 10ng/ μ l DNA template, 1.0 μ l of MgCl₂, 0.8 μ l of 10mM dNTPs, 4.6 μ l of ultra-pure water, 0.5 μ l of SSR primers, and 0.1 μ l Hot startTaq (Promega). The PCR reactions were loaded into the PTC-200 thermal cycler, and DNA was amplified using the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min, with a final extension of at 72°C for 5 min. The amplified PCR products were separated by electrophoresis on 3% agarose gels in 0.5XTBE buffer and stained with ethidium bromide. The gel was viewed and photographed under ultraviolet (UV) light using the GelDoc system. The unambiguous presence or absence of DNA fragments was scored as 1 or 0, respectively, and bands that could not be confidently scored were regarded as missing data.

Data Collection and Statistical Analysis

Table 3 summarizes the morphological traits measured. The meter rule was used to measure plant height. Seed length and width were measured using Vernier calipers, and seed weight was measured with an electronic weighing balance. The qualitative traits were scored visually. Variability among genotypes was estimated by clustering analysis using NTSYS-pc version 2 (Rohlf, 1998), and PCA using the Statistical Analysis System (SAS) package (version 9.4; SAS, 2013). Pairwise distance (similarity) matrices were computed using the SAHN clustering option of the NTSYS-pc software package version 2.02j; (Rohlf, 1998). The program generated dendrograms, which grouped the test lines based on Nei genetic distance (Nei, 1972) using the UPGMA cluster analysis (Sneath and Sokal, 1973).

The Additive Main Effect and Multiplicative Interaction (AMMI) model (Zobel *et al.*, 1988) was performed using MATMODEL 2.0 (Gauch and Furnas, 1991; Gauch, 1998). In the analysis, each combination between the location and a year was considered as an environment, therefore making a total of 6 environments for each ecology. The linear model for this analysis is as follows:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum \lambda_n Y_{gn} \eta_{en} + \theta_{ge} + \rho_{ge} + \epsilon_{ger}$$

where Y_{ger} is the trait rating of genotypes in environment e for replicate r , μ is the grand mean, α_g is genotype mean deviation (mean minus the grand mean), β_e is the environment mean deviations, N is the number of PCA axes retained in the model, λ_n is the singular value of PCA axis n , Y_{gn} is the genotype eigen vector values for PCA axis n , η_{en} is the environment eigen vector values for PCA axis n , θ_{ge} are the interaction residuals, ρ_{ge} is the AMMI residuals, and ϵ_{ger} is the residual error. The model uses the analysis of variance (ANOVA) approach to study the main effects of genotypes and environments, and a Principal Component Analysis (PCA) for the residual multiplicative interaction between genotypes and environments.

Table 3. Parameters measured in each of the rice genotype for the morphological characterization studies.

S/No	Character	Description
1	Seedling vigour	1=extra; 3=vigorous; 5=normal; 7=weak; 9=very weak
2	Basal leaf sheath colour	1=green; 2=purple line; 3=light purple; 4=purple
3	Plant height (Ht)	Actual measurement from soil surface to the tallest panicle (awn excluded)
4	Number of days to heading	Number of days from seeding to the heading of 50% of the plant on the plot.
5	Maturity	Number of days from seeding to the maturity of 85% of grains on panicle
6	Panicle exertion	1=well exerted; 3=moderately well exerted; 5=just exerted; 7=partly exerted; 9=enclosed
7	Leaf length	Actual length measurement (cm) of the leaf just below the flag leaf
8	Leaf width	Actual measurement (cm) of the widest portion of the leaf just below the flag leaf
9	Leaf angle	1=erect; 5=horizontal; 7=droopy
10	Number of tiller at 60 days	Actual count of total number of tillers per m ²
11	Panicle length	Actual measurements (cm) from panicle base to the tip
12	Panicle threshability	1=<1%; 3=1-5%; 5=6-25%; 7=26-50% 9=51-100%
13	Panicle shattering	1=<1%; 3=1-5%; 5=6-25%; 7=26-50% 9=51-100%
14	Awning	0=absent; 1=short & partly; 5=short & fully; 7=long & partly; 9=long & fully
15	Hairiness	0=absent; 1=slightly; 3=moderately; 5=highly
16	Primary panicles branching	0=absent; 1=light; 2=heavy; 3=clustering
17	Secondary panicles branching	0=absent; 1=light; 2=heavy; 3=clustering
18	Grain length	Mean length in mm as distance from the lower base of sterile lemma to the tip of the apiculus
19	Grain width	The measurement of the widest point along the grain
20	Grain yield	Weight of harvested grain per hectare at 14% moisture content
21	Number of Panicles per m ²	Actual count of the number of panicle m ²
22	1000-grain weight (g)	Measurement in grams of 1000 well developed whole grain

The Sites Regression analysis (SREG) linear-bilinear model is represented by

$$\bar{y}_{ij} = \mu + \delta_j + \sum_{k=1}^t \lambda_k \alpha_{ik} \gamma_{jk} + \bar{\epsilon}_{ij}$$

where \bar{y}_{ij} is the mean of the i th cultivar in the j th environment for g genotypes and e environments ($i = 1, 2, \dots, g$ and $j = 1, 2, \dots, e$); μ is the overall mean; δ_j is the site effect; λ_k ($\lambda_1 \geq \lambda_2 \geq \dots \geq \lambda_t$) are scaling constants (singular values) that allow the imposition of ortho normality constraints on the singular vectors for genotypes, $\alpha_k = (\alpha_{1k}, \dots, \alpha_{gk})$ and sites, $\gamma_k = (\gamma_{1k}, \dots, \gamma_{ek})$; α_{ik} and γ_{jk} for $k = 1, 2, 3, \dots$ are called "primary," "secondary," "tertiary," ... etc. effects of the i th genotype and

j th site, respectively; θ_{ij} is the residual error assumed to be normally and independently distributed $(0, \theta^2/r)$ (where θ^2 is the pooled error variance and r is the number of replicates). In the SREG model, the main effects of genotypes (G) plus the GE interaction were absorbed into the bilinear terms (Crossa *et al.*, 1991).

The GGE biplot methodology, which is composed of two concepts, the biplot concept (Gabriel, 1971) and the GGE concept (Gauch and Zobel 1996; Yan *et al.*, 2000), was also used to visually analyse the results of SREG analysis of MET data. This methodology uses a biplot to show the two factors (G plus GE) that are important in genotype evaluation and that are also the sources of variation in SREG model analysis of MET data (Yan *et al.*, 2000; Yan, 2001). The GGE biplot shows the first two principal components (PC1 and PC2, also referred to as primary and secondary effects, respectively) derived from subjecting environment-centered yield data (the yield variation due to GGE) to singular value decomposition (Yan *et al.*, 2000). In this study, GGE biplots were used to compare the performance of different genotypes at an environment, compare the performance of a genotype at different environments, compare the performance of two genotypes in all environments, identify the highest yielding genotypes at the different mega environments, and identify ideal cultivars and test locations.

Polymorphic information content (PIC) provided an estimate of the discriminatory power of a given locus or loci, by taking into account not only the number of alleles that were expressed but also their relative frequencies. PIC values were calculated using the following equation (Botstein *et al.*, 1980):

$$PIC = 1 - \sum_{i=1}^n P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^n 2P_i^2 P_j^2$$

where P_i^2 and P_j^2 represents the frequency of the i^{th} and j^{th} alleles, and n is the number of alleles

The population structure (Q) of the varieties was evaluated using STRUCTURE v2.3.4 software. The optimum number of populations was selected with a burn-in period of 100,000 steps followed by 100,000 MCMC (Monte Carlo Markov chain replicates). The range of genetic clusters was set from K = 1 to 10 with 10 iterations. To determine the true value for K, *ad hoc* statistic ΔK was calculated according to Evanno *et al.*, (2005).

Results

Genotype x Environment Interaction and stability of performance of Lowland NERICA.

The result of the combined analysis of variance for flowering days, maturity days, plant height, panicle/m² and yield of 48 rice genotypes at 12 environments is presented in Table 4. Significant replicate effects were observed for flowering days, maturity days, plant height, panicle/m² and yield. Also, the result indicates that the rice genotypes varied significantly for all traits. The location, genotype x location were highly significant to all traits except panicle/m². The two years differed significantly for all traits meaning that climatic changes were observed during the study. Significant genotype x year effects were observed for flowering days and maturity days, but non-significant G x E effects were observed for plant height, panicle/m² and yield meaning that the last three traits remained similar over the two years. Location x year interaction reported highly significant effects for all the five traits meaning that the location of experiments differed in the two years of the study.

Table 4. Mean squares of the combined analysis of variance for yield and related characters of rice genotypes at 12 environments (6-locations by 2-seasons).

Source	DF	Flowering days	Maturity days	Plant Height	Panicle/m ²	Yld (Kg)
Rep	2	405.57**	320.90*	935.73*	17802.79**	3172332.00*
Genotype	47	573.99**	445.64**	2510.16**	3224.97*	4241473.00**
Location	5	3415.67**	3293.76**	16468.86**	58159.07**	478999838.00**
Genotype x Location	235	39.00**	59.01**	431.84**	1640.01 ^{ns}	2045861.00**
Year	1	598.55**	987.06**	11891.26**	2518782.18**	214102592.00**
Genotype x Year	47	55.59**	74.22*	60.48 ^{ns}	1076.35 ^{ns}	1323782.00 ^{ns}
Location x Year	5	11053.14**	4314.64**	6147.59**	662751.43**	337035964.00**
Genotype x Location x Year	235	56.47**	48.14 ^{ns}	60.66 ^{ns}	1267.41 ^{ns}	1876652.00*
Error	1150	23.14	39.26	107.85	1895.73	1411702.00

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

This suggests that rice genotypes performed differently in every location in each year. Genotype x location x year was significant for days to flowering and yield and non-significant effects were observed for maturity days, plant height, and panicle/m².

Table 5 shows the mean of twenty-two characters measured in forty-eight rice genotypes in the six locations in two years. The highest mean grain yield of 4469.0Kg/ha was recorded in NERICA-L-28 while the lowest yield (2148.0Kg/ha) was observed in TOG 5681. The tallest genotype was NERICA-L-39 with a height of 123.0cm while NERICA-L-12 was the shortest with a height of 89.0cm. TOX 4004-43-1-2-1 had the highest number of panicles per meter square (219)

while NERICA-L-60 had the lowest (172). FKR 19 (Check) was the earliest maturing among the genotypes with the number of days to heading of 81 days, while SUAKOKO 8 was the latest to head with the number of days to heading of 101 days. The genotype with the earliest maturity was FKR 19 with the number of days to maturity of 112 days while the latest to mature was SUAKOKO 8 with a number of days to maturity of 129 days.

Table 5. The means of twenty-two characters measured in forty-eight rice genotypes in 12 environments (6-locations by 2-seasons)

40	NERICA-L60	92	121	93	12	172	3764	25.68	6
41	SUAKOKO 8	101	129	91	11	183	3618	26.21	5
42	TOX 4004-43-1-2-1	95	121	116	11	219	3766	27.53	5
43	WITA 7	92	123	106	11	184	3824	24.32	7
44	TOG 5681 (Parent)	85	114	108	12	181	2148	23.36	6
45	IR 64 (Parent)	84	116	93	13	189	3587	25.23	6
46	WITA 4 (Check)	94	124	112	12	206	4149	26.37	5
47	FKR 19 (Check)	81	112	102	11	196	3229	24.59	6
48	FKR 54 (Check)	83	116	108	10	202	4177	26.11	6
	Means	89	119	98	12	199	3864	25.93	6
	LSD	2.22	2.90	4.80	1.17	20.14	549.47	2.33	0.52

Plot/No	Pthres	Hairnes	Awning	Prybrpan	Secbrpan	Lflgth	Lfwidth	FlaglAng	Bastlcol	Grght	Grwidth
1	3	2	0	10	25	32.23	1.07	1	1	8.44	2.35
2	5	2	0	10	19	27.61	1.17	1	1	8.98	2.33
3	4	2	0	10	16	27.75	1.20	4	2	7.72	2.66
4	4	2	0	10	20	30.81	1.10	2	3	8.88	2.25
5	3	4	5	10	21	30.13	1.08	3	3	8.59	2.17
6	4	3	0	10	17	27.90	1.10	2	1	8.98	2.25
7	3	3	0	10	21	28.76	1.10	2	1	8.83	2.51
8	3	2	0	9	19	29.29	1.07	1	1	9.18	2.45
9	5	2	0	9	17	27.01	1.10	1	3	8.72	2.29
10	3	2	0	9	18	29.43	1.08	1	1	9.14	2.24
11	3	4	0	10	19	26.00	1.03	1	1	8.75	2.33
12	4	4	0	8	15	27.25	1.05	2	3	9.43	2.30
13	3	2	0	10	20	29.88	1.04	1	1	9.43	2.30
14	3	2	0	11	21	30.81	1.07	1	1	9.55	2.29
15	4	2	0	11	19	27.92	1.06	1	1	9.27	2.24
16	3	2	0	8	17	27.94	1.08	1	1	8.72	2.18
17	4	2	0	10	21	32.59	1.03	2	1	9.20	2.30
18	4	2	0	10	23	30.25	1.06	1	1	9.30	2.32
19	5	2	0	10	25	28.32	1.00	1	1	8.87	2.55
20	3	3	0	10	21	28.01	1.05	3	1	8.65	2.28
21	5	2	0	8	17	28.68	1.02	1	1	8.86	2.25
22	5	2	0	9	17	27.11	1.03	1	1	9.25	2.35
23	4	2	0	10	19	29.14	1.01	1	1	9.06	2.25
24	4	2	0	9	19	26.65	1.08	1	3	8.54	2.21
25	3	3	0	10	21	29.49	1.08	1	1	8.77	2.27
26	4	2	0	9	20	28.67	1.05	1	1	8.54	2.40
27	3	2	0	10	23	33.75	0.93	3	3	8.29	2.14
28	3	2	0	9	18	28.21	1.09	2	1	8.96	2.28
29	4	2	0	10	22	30.75	1.11	1	1	8.42	2.28
30	4	2	0	10	22	30.81	1.02	1	1	8.93	2.36

31	4	2	0	9	19	27.74	1.09	1	1	8.82	2.39
32	3	3	0	9	20	29.12	1.00	1	1	9.02	2.44
33	4	2	0	9	19	29.68	1.03	1	1	8.88	2.24
34	4	2	0	11	22	30.08	1.04	1	1	8.80	2.44
35	3	2	0	9	17	26.65	1.01	1	1	9.39	2.34
36	4	2	0	9	18	29.64	1.01	1	1	8.71	2.32
37	4	3	0	9	17	27.09	1.04	1	3	8.86	2.25
38	3	2	0	9	21	30.07	1.03	2	3	9.74	2.40
39	4	1	0	10	21	30.10	1.06	1	1	8.57	2.29
40	3	3	0	10	21	29.82	1.07	1	1	8.68	2.33
41	3	2	0	11	21	33.98	0.95	2	3	8.85	2.31
42	5	2	1	11	25	30.48	1.01	2	1	9.07	2.52
43	5	2	0	11	20	28.43	1.00	2	1	8.54	2.45
44	3	2	0	9	15	26.59	1.04	3	3	8.00	2.59
45	4	3	0	10	21	26.86	0.93	1	1	8.57	2.36
46	5	2	0	10	20	28.87	0.97	3	1	8.73	2.31
47	3	2	0	9	19	26.07	0.93	2	1	8.38	2.32
48	4	2	0	10	21	29.74	1.14	2	1	8.65	2.45
Mean	4	2	0	10	20	29.05	1.05	2	1	8.84	2.34
Lsd	0.00	0.16	0.00	0.49	2.29	2.17	0.08	0.30	0.09	0.15	0.05

Genotype x environment interaction and stability analysis

The additive main effect and multiplicative interaction (AMMI) analysis of variance for seed yield per plot in forty-eight genotypes tested across 12 environments (6-locations by 2-seasons) (Table 6). The result showed strong evidence that environment (E), genotype (G), and genotype-by-environment (G x E) interaction were highly significant at ($p < 0.01$), as E and G, respectively accounted for 78.6, 3.5, and 17.9% of the total variation. The total sum of squares due to G x E interaction was mainly explained by the first two principal component axes (IPCA1 and 2), which were significant and respectively accounted for 27.3% and 23.8% of the sum squares. The IPCA1 mean square was almost four times larger than the error means square. The IPCA 3 and IPCA 4 were equally significant and accounted for 13.3% and 10.5% of the G x E interactive sum of squares, respectively. Table 7 shows the GGE analysis of variance for rice yield in forty-eight rice genotypes evaluated across twelve environments (6-location by 2-seasons). The result showed significant ($P < 0.01$) Environment (E), Genotype (G), and Genotype-by-Environment (G x E) interaction that accounted for 60.4%, 2.8%, and 13.8% of the total sum squares, respectively. The environmental sum of squares was about twenty-one times larger than the genotype sum of squares and four times larger than the GEI sum of squares.

The biplot of AMMI for 48 rice genotypes in 12 environments. The y-axis represents the IPCA1 scores, while the x-axis represents the seed yield per plot (main effect) of the accessions (Figure 3). NERICA-L56 was the overall best genotype combining relative stability and high yield. Genotypes NERICA-L8, NERICA-L12, NERICA-L33, NERICA-L36, NERICA-L42, and FKR 54 were highly stable and above average in yield, while NERICA-L28 was above average in yield but relatively unstable due to large interaction. IR 64 and NERICA-L60 had a subpar yield but stable. The poorest of the genotypes due to instability and lowest yield were TOG 5681 and FKR 19. Irrigated Ibadan 2008 (E1), Irrigated Ibadan 2009 (E2), Valley bottom Ibadan 2008 (E3), Valley fringe Ibadan 2008 (E5), Valley fringe Ibadan 2009 (E6), and Valley fringe Kpalime 2009 (E8) had subpar yield. The valley bottom Ouédémé 2008 (E9) and valley fringe Ouédémé 2008 (E11) were most stable whereas valley bottom Ouedeme 2009 (E10) and valley fringe Ouédémé 2009 (E12) were most unstable producing large interactions. Environment (E7) was observed to be next to E9 as far as yield and stability of performance are concerned.

Table 6: The additive main effect and multiplicative interaction (AMMI) model analysis of variance for rice yield in forty-eight genotypes tested across 12 environments (6-locations by 2-seasons).

Source	Df	Sum of squares	Mean square	Percentage total Sum of squares	Percentage treatment	Percentage G x E
Total	1726	7097293236.77	4111989.13			
Treatment	575	5471072240.27	9514908.24**	77.1		
Genotype	47	192681506.17	4099606.51**		3.5	
Environment	11	4299546982.94	390867907.54**		78.6	
G x E	517	978843751.17	1893314.80**		17.9	
IPCA 1	57	267371718.91	4690731.91**			27.3
IPCA 2	55	232488363.78	4227061.16**			23.8
IPCA 3	53	130469255.67	2461684.07**			13.3
IPCA 4	51	102507705.53	2009955.01*			10.5
IPCA 5	49	80847056.65	1649939.93			8.3
IPCA 6	47	70716162.42	1504599.20			7.2
IPCA 7	45	40623835.32	902751.90			4.2
Residual	160	53819652.89	336372.83			
Error	1151	1626220996.50	1412876.63	22.9		

*, **, significant at 5% and 1% probability level respectively

Table 7: GGE analysis of variance for rice yield in forty-eight rice genotypes evaluated across twelve environments (6-location by 2-seasons).

Source	Df	SS	MS	% Total SS
Total	1727	7107535378.46		
Genotype	47	199353914.53	4241573.00**	2.8
Environment	11	4294297637.53	390390700.00**	60.4
G x E	517	984022532.68	1903332.00**	13.8
Block	24	351823964.70	14659330.00**	
Error	1128	1278037329.02	1133012.00	

*, **, significant at 5% and 1% probability level respectively

Correlation Among the Morphological Traits of Rice Genotypes

All 48 rice genotypes were evaluated in 12 environments (Table 8). Plant vigor was negatively and highly significantly correlated with plant height ($r = -0.55$), panicle number per square meter (-0.56), primary branch panicle (-0.33), secondary branch panicle (-0.30), and leaf length (-0.50). Tiller number per square meter showed a highly significant negative correlation with plant height (-0.35) and primary branch panicle number (-0.29%), and a significant negative correlation with grain length (-0.19) and grain width (-0.20). Days to flowering showed a highly significant positive correlation with maturity date (0.97), plant height (0.65), panicle number per square meter (0.27), panicle length (0.35), primary branch panicle (0.58), secondary branch panicle (0.42), and leaf length (0.75); significant positive correlation with panicle threshability (0.16) and grain width (0.17); highly significant negative correlation with panicle exertion (-0.34); and significant negative correlation with hairiness (-0.17). Days to maturity had a highly significant positive correlation with plant height

Table 8: The genotypic correlation coefficient between twenty-two characters of rice in twelve environments (6 locations by 2 seasons)

Character	No	Flw	Mat	Plt	Pan	PSht	Pthres	Yld	Hairnes	Pan/	Awn	Pan	Pry	Sec	Lf
	Till	days	days	Hght	Ext					meter		lght	brpan	brpan	lght
Pltvigor	0.04	0.04	-0.06	-0.55**	-0.11	0.04	-0.11	0.14	0.15	-0.56**	-0.15	0.04	-0.33**	-0.30**	-0.50
NmTiller		0.12	0.12	-0.35**	-0.09	-0.07	-0.08	-0.14	0.06	0.02	-0.04	-0.14	-0.29**	-0.05	-0.02
Flwdays			0.97**	0.65**	-0.34**	-0.04	0.16*	0.03	-0.17*	0.27**	0.12	0.35**	0.58**	0.42**	0.75*
Matdays				0.66**	-0.26**	-0.05	0.15	0.09	-0.14	0.56**	0.15	0.43**	0.63**	0.54**	0.87*
PltHght					-0.11	-0.09	0.19*	-0.27**	-0.28**	0.67**	-0.01	0.26**	0.68**	0.45**	0.74*
PanExt						-0.13	-0.12	0.30**	-0.06	-0.01	-0.04	-0.27**	0.17	-0.01	-0.31
PSht							0.20*	0.45**	-0.27**	-0.19*	-0.18*	0.28**	-0.05	0.05	-0.12
Pthres								-0.04	-0.18*	0.40**	0.02	0.15	0.16*	0.17*	0.27*
Yld									-0.08	-0.34**	0.01	0.79**	0.24**	0.26**	0.08
Hairnes										-0.21**	0.46**	-0.55**	-0.18*	-0.24**	-0.36
Pan_m											-0.45**	0.38**	0.80**	0.46**	0.87*
Awning												0.04	0.13	0.19*	0.14
Panlght													0.48**	0.67**	0.94*
Prybrpan														0.79**	0.65*
Secbrpan															0.74*
Lflgth															
Lfwdth															
Lfwdth															
Bastlcol															
Grlght															
Grwidth															

(0.66), panicle number per square meter (0.56), panicle length (0.43), primary branch panicle (0.63), secondary branch panicle (0.54), and leaf length (0.87), and highly significant negative correlation with panicle exertion (-0.26). Plant height showed a highly significant positive correlation with panicle number per square meter (0.67), panicle length (0.26), primary branch panicle (0.68), secondary branch panicle (0.45), leaf length (0.74), and grain width (0.23); significant positive correlation with panicle threshability (0.19); and highly significant negative correlation with yield (-0.27) and grain length (-0.27).

Panicle exertion showed a highly significant positive correlation with yield (0.30). Meanwhile, panicle exertion showed a highly significant negative correlation with panicle length (-0.27), leaf length (-0.31), basal leaf sheath coloration (-0.33), and significant correlation with 1,000-grain weight (-0.17). Panicle shattering showed a highly significant positive correlation with yield (0.45) and panicle length (0.28); significant correlation with panicle threshability (0.20); highly significant negative correlation with hairiness (-0.27); and significant correlation with (-0.18). Panicle threshability showed a significant negative correlation with hairiness (-0.18); highly significant positive correlations were panicle number per square meter (0.40) and leaf length (0.27); and significant correlation with primary branch panicle (0.16) and secondary branch panicle (0.17). Yield showed a highly significant positive correlation with panicle length (0.79), primary branch panicle (0.24), secondary branch panicle (0.26), grain length (0.37), and 1,000-grain weight (0.24), and highly significant negative correlation with panicle number per square meter (-0.34) and basal leaf sheath coloration (-0.41).

Hairiness showed a highly significant positive correlation with awn (0.46) and basal leaf sheath coloration (0.23); highly significant negative correlation with panicle number per square meter (-0.21), panicle length (-0.55), secondary branch panicle (-0.24), and leaf length (-0.36); and significant negative correlation with primary branch panicle (-0.18). Panicle number per square meter was a highly significant negative correlation with awning (-0.45) and grain length (-0.55), and highly significant positive correlation with panicle length (0.38), primary branch panicle (0.80), secondary branch panicle (0.46), and leaf length (0.87). Awning showed a highly significant positive correlation with basal leaf sheath coloration (0.24); a significant positive correlation with secondary branch panicle (0.19); and a significant negative correlation with grain width (-0.18). Panicle length showed a highly significant positive correlation with primary branch panicle number (0.48), secondary branch panicle number (0.67), and leaf length (0.94), and significant positive and negative correlation with grain length (0.17) and grain width (-0.53), respectively.

Primary branch panicle number showed a highly significant positive correlation with secondary branch panicle (0.79), leaf length (0.65), and grain width (0.23), and highly significant negative correlation with basal leaf sheath coloration (-0.25) and 1,000-grain weight (-0.25). Secondary branch panicle number showed a highly significant positive correlation with leaf length (0.74) and a highly significant negative correlation with basal leaf sheath coloration (-0.30). Leaf length showed a highly significant positive correlation with grain width (-0.22) and a significant positive correlation with grain length (0.18). Leaf width showed a highly significant positive correlation with grain width (0.33), 1,000-grain weight (0.34), basal leaf sheath coloration (0.35), and grain width (0.25),

and a highly significant negative correlation with grain length (-0.58). Highly significant negative correlations were also observed between basal leaf sheath coloration and grain length (-0.21), grain length and grain width (-0.34), and grain width and 1,000-grain weight (-0.25).

Variability Among 48 Rice Genotypes Based On Morpho-agronomic Traits

PCA of the morpho-agronomic traits of 48 rice genotypes revealed eight PC axes with Eigenvalues greater than 1.0, which together accounted for 75.13% of the total variation. The relative discriminating power of the PCA, as revealed by Eigenvalues, was 4.13, 2.77, 2.36, 1.83, 1.66, 1.43, 1.27, and 1.09 for PC1, PC2, PC3, PC4, PC5, PC6, PC7, and PC8, respectively. PC1, PC2, PC3, and PC4 explained 18.79%, 12.57%, 10.71%, and 8.33% of the total variation, respectively, together accounting for 50.39% of the total variation. PC1 attributed to variation in days to flowering, days to physiological maturity, plant height, primary branch panicle, secondary branch panicle, and leaf length. PC2 was associated with yield, panicle shattering, panicle threshability, flag leaf angle, and base tiller coloration. PC3–6, and PC8 mainly attributed to variation in grain width, awning, panicle exertion, plant vigor, and panicle number per square meter, respectively, whereas PC7 was associated with tiller number at 60 days (Table 9). The plot of the relationship among all 48 genotypes showed a considerable amount of variability, although most of the genotypes from the same source did not sort out, as explained by PC1 and PC2 (Figure 4). Additionally, the results of PCA showed that rice genotypes were grouped into four distinct clusters. The ordination of rice genotypes on PC1 and PC2 accounted for 51.5% of the total variation, showing that genotypes TOG 5681, FKR 19, WITA 4, NERICA-L38, and NERICA-L28 were quite distinct from other genotypes (Figure 3).

Table 9: Phenotypic characteristics with respect to their principal component, Eigen values and variation of 48 rice genotypes.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
Flowering date	0.79*	-0.11	0.08	0.2	0.07	-0.31	0.31	-0.18
Maturity date	0.84*	-0.09	0.15	0.18	0.05	-0.21	0.24	-0.18
Plant height (cm)	0.68*	-0.14	-0.32	-0.17	-0.03	0.25	-0.01	0.27
Number of Tiller at 60 days	-0.15	0.03	0.26	0.03	-0.12	-0.26	0.58*	0.39
Panicle number/m ²	-0.19	0.1	0.15	0.42	0.17	0.16	0.11	0.64*
Yield (g)	0.12	0.64*	0.3	0.21	0.41	0.2	0.14	0
Panicle length (cm)	0.39	0.43	0.24	0.04	-0.18	0.35	-0.07	-0.15
Panicle exertion	-0.09	-0.06	-0.13	-0.54	0.64*	0.11	-0.1	-0.16
Plant vigour	-0.32	0.15	0.17	0.38	0.22	-0.58*	0.08	-0.29
Panicle shattering	0.02	0.69*	-0.57	0.37	-0.12	0.02	-0.07	-0.06
Panicle threshability	0.02	0.69*	-0.57	0.37	-0.12	0.02	-0.07	-0.06
Hairnes	-0.26	-0.41	0.2	0.35	0.46	-0.1	-0.25	0.01
Awning	0.14	-0.31	0.19	0.52*	0.32	0.27	-0.23	0.05
Primary branch panicle	0.77*	0.15	-0.04	-0.09	0.34	-0.1	-0.22	-0.05
Secondary branch panicle	0.71*	0.24	0.26	-0.12	0.06	-0.1	-0.2	0.28
Leaf length (cm)	0.77*	0.01	0.41	-0.02	-0.24	0.05	-0.04	-0.01
Leaf width (cm)	-0.06	0.1	-0.1	0.03	0.47	0.5	0.59*	-0.2
Flag leaf angle	0.37	-0.60*	-0.39	0.29	0.03	0.24	0.02	0.06
Base tiller coloration	-0.02	-0.52*	-0.06	0.5	-0.25	0.18	-0.05	-0.2
Grain length (mm)	-0.19	0.41	0.49	0	0.15	-0.03	-0.3	0.08
Grain width (mm)	0.14	-0.05	-0.61*	-0.19	0.27	-0.25	0.17	0.18
1000grain weight (g)	-0.2	0.04	0.45	-0.15	-0.24	0.35	0.22	-0.2
Eigen value	4.13	2.77	2.36	1.83	1.66	1.43	1.27	1.09
% variance	18.8	12.57	10.71	8.33	7.55	6.5	5.76	4.93
Cumulative % variance	18.8	31.36	42.07	50.4	57.9	64.44	70.2	75.1

*component contributors; PC: Principal component

Furthermore, 48 rice genotypes were delineated into nine distinct clusters using the FASTCLUS clustering procedure (Table 10). Clusters I–IX contained 8, 2, 9, 2, 1, 9, 10, 6, and 1 varieties, respectively. Genotypes in cluster II (19 and 2) out yielded the checks with the highest yield (4,181 g) and longest panicle (30.06 cm), while genotypes in cluster VI (6, 27, 32, 34, 37, 39, 40, 44, and 46) showed the lowest grain yield (3,689 g). Compared with other genotypes, those in cluster IV (4 and 45) were early in flowering (by 85 days) and reaching 70% physiological maturity (by 114 days). The genotype in cluster V (26) showed the

highest plant height at maturity. Genotypes in clusters I and IX (1, 18, 22, 24, 25, 30, 35, 36, and 48) showed the highest tiller number, while the accession in cluster V (26) showed the lowest tiller number and panicle exertion. Panicle number per square meter was the highest in cluster III genotypes (3, 14, 16, 21, 28, 38, 41, 43, and 47) and lowest in cluster IV genotypes (4 and 45). Genotypes in clusters IV (4 and 45) and V (26) showed the lowest plant vigor, and together with the genotype in cluster IX (3), the lowest panicle shattering. The genotype in cluster V (26) showed greater panicle threshability than those in cluster II (19 and 2). Genotypes in clusters VII and IX (7, 8, 9, 10, 11, 15, 20, 24, 29, 31, and 42) showed pronounced hairiness.

Table 10: Mean and standard of deviation of characters that separate the 48 rice genotypes into nine distinct clusters using the FASTCLUS clustering procedure.

Character (Group)	I	II	III	IV	V	VI	VII	VIII	IX	Min	Max
	1, 18, 22	19, 2	14, 16, 21	4, 45	26	6, 27, 32	7, 8, 9, 10	5, 12, 13	24		
	25, 30, 35		28, 3, 38			34, 37, 39	11, 15, 20	17, 23, 33			
	36, 48		41, 43, 47			40, 44, 46	29, 31, 42				
Flowering days	90(2.6)	87(0.4)	90(4.4)	85(0.0)	87(8.9)	91(4.9)	89(4.9)	87(3.9)	92(2.7)	85	9
Maturity days	119(3.1)	117(1.3)	120(3.2)	114(0.0)	118(8.9)	120(4.4)	119(3.6)	118(2.1)	123(0.0)	114	1
Plant height (cm)	97(7.7)	94(1.9)	102(7.0)	108(0.0)	113(15.1)	101(11.1)	100(7.7)	102(4.7)	93(0.0)	93	1
Number of tiller	13(1.5)	12(0.8)	12(0.9)	12(0.0)	11(0.4)	12(0.7)	12(0.5)	12(1.0)	13(0.0)	11	1
Panicle/m ²	198(8.8)	190(0.3)	203(4.5)	181(0.0)	194(3.1)	198(15.4)	200(9.3)	199(7.2)	200(0.0)	181	2
Yield (gms)	3881(35.9)	4181(23.3)	3772(41.2)	3803(0.0)	380889.0)	3689(27.7)	3963(50.4)	33965(51.5)	4094(0.0)	3689	4
Panicle length	25.43(1.0)	30.06(5.5)	25.72(0.9)	23.36(0.0)	26.86(3.2)	26.04(0.9)	25.63(1.1)	25.96(0.5)	26.14(0.0)	23.36	3
Panicle Exertion	6(0.6)	6(0.0)	6(0.5)	6(0.0)	5(0.8)	6(0.4)	6(0.5)	6(0.5)	6(0.0)	5	6
Plant vigor	3(0.5)	3(0.2)	3(0.4)	2(0.0)	2(0.1)	3(0.3)	3(0.4)	3(0.3)	3(0.0)	2	3
Panicle Shattering	4(0.4)	4(1.0)	4(0.5)	3(0.0)	3(0.1)	4(0.6)	4(0.8)	4(0.7)	3(0.0)	3	4
Panicle Threshability	5(1.5)	4(0.5)	5(1.7)	5(0.0)	6(0.9)	5(1.0)	5(1.0)	6(1.6)	5(0.0)	4	6
Hairness	2(0.3)	2(0.7)	2(0.8)	2(0.0)	2(0.0)	2(0.4)	3(0.9)	2(0.0)	3(0.0)	2	3
Awning	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.3)	1(1.6)	0(0.0)	0(0.0)	0	1
Primary branch panicles	10(0.8)	10(0.3)	10(0.6)	9(0.0)	10(0.7)	10(0.8)	9(0.9)	10(0.7)	10(0.0)	9	1
Secondary branch panicles	20(2.9)	20(1.3)	19(2.0)	15(0.0)	21(2.5)	20(2.4)	19(2.4)	21(1.5)	21(0.0)	15	2
Leaf length (cm)	28.91(1.6)	27.81(0.3)	28.68(2.1)	26.59(0.0)	29.91(5.4)	29.45(2.2)	29.08(1.2)	29.57(1.5)	29.49(0.0)	26.59	2
Leaf width (cm)	1.03(0.0)	1.11(0.1)	1.07(0.1)	1.04(0.0)	0.93(0.0)	1.02(0.1)	1.06(0.0)	1.07(0.0)	1.08(0.0)	0.93	1
Flag Angle	1(0.1)	2(1.4)	2(1.0)	3(0.0)	2(0.7)	2(0.2)	2(0.7)	1(0.2)	1(0.0)	1	3
Base tiller coloration	1(0.8)	1(0.0)	1(0.8)	3(0.0)	2(1.8)	1(1.0)	2(1.0)	1(0.9)	1(0.0)	1	3
Grain length (mm)	8.73(0.2)	8.82(0.2)	8.74(0.5)	8(0.0)	8.34(0.1)	9.04(0.4)	8.94(0.2)	9.05(0.4)	8.77(0.0)	8	9
Grain width (mm)	2.37(0.1)	2.31(0.0)	2.34(0.1)	2.59(0.0)	2.23(0.1)	2.36(0.1)	2.3(0.1)	2.34(0.1)	2.27(0.0)	2.23	2
1000gwt (gms)	23(0.6)	23(0.1)	24(0.7)	23(0.0)	24(0.2)	23(1.1)	24(0.7)	24(0.8)	21(0.0)	21	2

Genotypes in cluster VII (7, 8, 9, 10, 11, 15, 20, 29, 31, and 42) showed prominent awnings, which were absent in other clusters. Genotypes in clusters IV (4, 45) and VII (7, 8, 9, 10, 11, 15, 20, 29, 31, and 42) showed fewer primary branch panicles (9) than those in other clusters (10). The highest number of secondary branch panicles (21) was found in varieties in clusters V (26), VIII (5, 12, 13, 17, 23, and 33), and IX (24), whereas genotypes in other cluster produced only 15-

20 secondary branch panicles. The genotype in cluster V (26) produced the longest leaf (30cm), whereas genotypes in cluster IV (4 and 45) showed the shortest leaf (25.6cm). Genotypes in clusters II (19 and 2) showed greater leaf width (1.1cm) than the genotype in cluster V (26) (0.93cm). Genotypes in cluster IV (4 and 45) showed the largest flag leaf angle and the most prominent base tiller coloration. Genotypes in nearly all nine clusters showed no significant differences in grain length (8–9 cm) and grain width (2.3–2.6 cm). However, genotype 24 in cluster IX recorded the lowest 1,000-grain weight (21.0g) compared with genotypes in other clusters (23 and 24g).

Marker performance and characterization

Of the 50 SSR markers used to genotype the 48 rice genotypes, 10 were polymorphic (Table 11). The study showed that makers used generated 49 alleles with an average of 4.9 alleles per marker. Alleles per marker ranged between 3 and 8 alleles. The highest number of alleles was observed with RM125 (Na = 8 alleles) while the least number of alleles were observed with EM433, RM494, and RM514 (NA = 3 alleles). The markers' ability to detect heterozygosity varied from marker to marker, RM433 detecting the highest heterozygosity (H = 0.93) and RM495 detecting the least heterozygosity (H = 0.02) among the samples evaluated. The polymorphic information content also varied among markers. RM154 had the highest PIC while the PIC was observed with RM162.

Genetic diversity and structure among rice samples

Structure analysis revealed a peak at $\Delta K = 44.22$ corresponding to $K=2$ (Figure 5a). This implied a two-substructure level with admixtures among the studied accessions. It was observed that the two parents did not fall within the same genetic structure, likewise the checks used in the study. A total of 16 samples comprising, 8 intraspecific, 6 interspecific, and 2 checks make the first genetic structure (Q1) while a total of 17, comprising majorly the interspecific samples (16) and a parent (IR64) make up the second genetic structure (Q2). Admixture categories comprised of 2 intraspecific, 11 interspecific, 1 check (FKR54), and 1 parent (TOG5681) (Figure 5b).

The study showed more alleles observed within the interspecific varieties than the intraspecific rice genotypes. Observed heterozygosity was lesser than expected in both genotype groups, although higher diversity was observed in the interspecific genotypes than the intraspecific genotypes. However, the fixation index was lower in the interspecific genotypes when compared to the intraspecific genotypes (Table 11). Analysis of molecular variance revealed that within-group variation was higher than variation observed between groups (Table 12).

Table 11. Population diversity among sample rice varieties.

Population	Na	Ne	I	Ho	He	F
Intraspecific						
Mean	3.40	2.22	0.88	0.46	0.53	0.19
SE	0.48	0.22	0.11	0.13	0.05	0.22
Interspecific						
Mean	3.80	2.26	0.90	0.45	0.52	0.17
SE	0.47	0.23	0.10	0.12	0.05	0.22
Total						
Mean	3.60	2.24	0.89	0.45	0.53	0.18
SE	0.33	0.16	0.07	0.09	0.04	0.15

Na: number of alleles; Ne: number of effective alleles; I: Shannon information's index; Ho: Observed heterozygosity; He: Expected heterozygosity; F: Fixation index

Table 12. Analysis of Molecular Variance.

Source	df	MS	Est. Var.	%Pol
Among Pops	1	16.397	0.646	9%
Within Pops	41	6.480	6.480	91%
Total	42		7.126	100%

MS; mean square; Est Var: estimated variance explained.

Clustering of rice genotypes

The dendrogram of 43 genotypes, their two parents, and three checks resulted in five clustered groups and one of the parents as an outgroup (TOG 5681) to the clusters at a similarity index of 0.49. The first cluster comprised of two intraspecific genotypes (1,3), the second cluster encompasses a majority of the genotypes distributed into four subclusters which include cluster IIA comprising of three intraspecific genotypes (2,4,7), cluster IIB comprised of six genotypes that are both intraspecific and interspecific (5, 6, 15, 16, 17, 19), likewise cluster IIC comprised of twenty genotypes (8, 9, 10,11, 18, 24, 28, 36, 40, 35, 37, 38, 39, 27, 23, 45, 30, 33, 34, 12) while are all interspecific genotypes except genotype 45 which represents one of the parents, IR64, while the last subcluster, IID,

comprised of three genotypes (20, 29, 42) that are interspecific in nature. The third cluster is composed of four interspecific genotypes (13, 14, 25, 26). The fourth cluster is comprised of six genotypes (21, 32, 22, 31,46, 48) that are both interspecific and checks. The fifth cluster is composed of the remaining three genotypes (41, 47, 43) which represent an interspecific genotype and two checks (Figure 6).

Discussion

The characterization and quantification of genetic diversity within closely related crop germplasm have long been a major goal of breeding programs, as it is essential for the rational use of genetic resources. Additionally, analysis of genetic variation among breeding materials is of fundamental interest to plant breeders, as it contributes immensely to the selection and monitoring of germplasm and the prediction of potential genetic gains (Chakravarthy and Rambabu, 2006). The genetic diversity of crop germplasm is heavily influenced by environmental factors. Morphological characterization of plants is inexpensive and serves as a valuable analysis tool in preliminary studies. However, DNA-based molecular markers have proven to be more powerful tools in the assessment of genetic variation and the elucidation of genetic relationships within and among species, and are untouched by environmental influence (Powell et al., 1996).

A high level of similarity was revealed between the NERICAs for most of the characters studied. The highest yield recorded in the 12 environments was (4469.00g/plot) for NERICA-L-28. There was significant variability amongst the genotypes as well as the environments as revealed by the analysis of variance, while the combined analysis of variance showed a significant genotype x environment interaction for all the characters evaluated, suggesting that all the characters responded differently to the different environments.

PCA reveals the importance and contribution of each component to the total variance and can be used to measure the independent contribution of a particular trait to the total variance. However, each coefficient of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated. The higher the correlation coefficient (positive or negative), the more effective in discriminating varieties (Sanni et al., 2012; Nachimuthu et al., 2014). In the current study, the results of PCA showed that PC1–8 was responsible for 75.13% of the total variation, and PC1–4 explained 50.39% of the total variation, accounting for most of the variability observed among the rice genotypes from different locations. This corroborates the findings of Nachimuthu et al. (2014) on rice germplasm collections from different locations. In addition, some of the morpho-agronomic traits such as days to flowering, days to physiological maturity, plant height, panicle number, grain yield, panicle exertion, panicle shattering, panicle threshability, primary branch panicle, secondary branch panicle, leaf length, flag leaf angle, and grain width were considered as the major contributors to the total variation, with PC values >0.6 in this study. Matus et al. (1999) and Akinyosoye et al. (2017) previously reported that PC values >0.6 could be regarded as major contributors to the total variation. This is consistent with the findings of Ray et al. (2013), who showed that grain characteristics, panicle density, leaf length, and plant height contribute to phenotypic diversity in Indian aromatic and non-aromatic rice landraces. Similarly, Caldo et al. (1996) reported that maturity, plant height, leaf length, and tillering ability are the major contributors to the variation among parental lines of modern Philippine rice cultivars. Therefore, the morpho-agronomic traits investigated in this study could facilitate the effective selection in rice breeding programs.

Multilocational trials are necessary to confirm the distinctiveness, uniformity, and stability of newly developed crop varieties for recommendation to researchers and farmers. The interaction that exists between genotypes and environment in diverse environments makes the selection of any genotype for recommendation a little challenging for breeders. Hence, there is a need to select for distinctiveness, uniformity, and stability, whenever such interactions become of practical value in a testing programme (Funnah and Mak, 1980). Thus, the Additive Main effect and Multiplicative Interaction (AMMI) analysis of variance for the forty-eight rice genotypes evaluated over twelve environments showed strong evidence that environment, genotype, and genotype x environment interaction were highly significant at $P \leq 0.01$, and accounted for 78.6, 3.5, and 17.9% of the total treatment sum of squares, respectively, suggesting that the forty-eight rice genotypes and the environments in which they were evaluated were significantly different from one another. The G x E interaction implicated the first interaction principal components axis (IPCA 1), which was significant and could account for most of the G x E interaction.

The above suggests that the climatic and soil conditions of the various environments interfered with the performance of the genotypes, especially since the IPCA 1 axes of the AMMI model usually relates to the length of the growing environment, temperature changes, variation in soil, or a combination of all factors and maturity group of the genotype (Gauch and Furnas, 1991). The result of AMMI revealed that NERICA-L8, NERICA-L12, NERICA-L33, NERICA-L36, NERICA-L42, and FKR 54 were the most stable genotypes because they had very little interaction with the environments as indicated by their IPCA scores of zero or near zero. Therefore, these genotypes can be cultivated in any of the 12 environments for their stability. NERICA-L28 was above average in yield but had high interactions, indicating that they were unstable and responsive to changes in the environment. IR 64 and NERICA-L60 had a subpar yield but stable. The poorest of the genotypes due to instability and lowest yield was TOG 5681 and FKR 19 and as such, they would require special attention to be able to perform well.

NERICA-L12, NERICA-L33, NERICA-L42, and NERICA-L56 appeared to be the overall best of genotypes, combining high stability with yield, therefore can be recommended for cultivation in any of the environments for high yield and stability. Genotypes with large interaction with the environment are unpredictable in performance and can only be grown in limited environments. Of the twelve environments, valley bottom Ouedeme 2008 (E9) produced the least interaction effect followed by valley fringe Ouedeme 2008 (E11) and maybe the most appropriate environments for rice production and evaluation. Selection within these environments will be effective as the relative performance and stability of these genotypes could be predicted with certainty.

Similarly, the GGE ANOVA also revealed a significant G x E interaction at $P \leq 0.01$ and accounted for 51.5, 31.3, and 20.2% of the total treatment sum of squares. This is also an indication that the environment recorded some level of interference with the genotype performance, hence may ultimately affect the stability of the performance of the genotype for the characters considered in this study. According to Yan and Kang (2002), the discriminating power and the average environment coordinate view of the GGE biplots was more effective in the evaluation of test environments relative to AMMI biplots. Concerning the ideal genotype and the average environment coordinate as indicated by the GGE biplot, NERICA-L8 was the best (most ideal) genotype, NERICA-L7, NERICA-L20, NERICA-L12, and FARO 51(CISADANE) were the most stable and closet to the ideal genotype. This suggests that these genotypes were better in yield and

were more stable than all the other genotypes in all environments studied. They would therefore be suitable for recommendation in the 12 environments. Furthermore, FARO 44 (SIPI-692033), NERICA-L14, and NERICA-L28 were found to be high-yielding but very unstable. However, TOG 5681 and FKR 19 have no place as far as yield and stability are concerned. Their poor yield may be associated partly with the G x E interaction as well as the poor genetic capacity of these genotypes. Although the two genotypes appeared promising in breeding for earliness, they may not be the choice for recommendation in respect to overall performance. Environments 9 and 11 (E9 and E11) were the closest to the ideal environment and can be considered the best environments for rice cultivation partly because they produced little interaction with the genotypes compared to the other environments and because there was the right temperature and low rainfall which reduced the magnitude and activities of pathogens.

According to the current study, both AMMI and GGE biplots identified two common genotypes NERICA-L8 and NERICA-L12 that were overall best in performance for yield and stability. This observation suggests that for reliability and optimum result, it is better to combine the result of the two analytical tools for yield and stability in the recommendation of genotypes to farmers. Therefore, NERICA-L12, NERICA-L33, NERICA-L42, and NERICA-L56 have a better prospect to perform better with high stability across the 12 environments. Between locations similarities and within location differences in rainfall pattern as well as the performance of crop genotype according to (Van Eeuwijk and Elgersma, 1993) suggest that climatic information might be useful in the clarification of genotype by trial interaction.

A biplot showed considerable variability among the 48 rice genotypes, although most of the genotypes with the same genetic background could not be distinguished by PC1 and PC2, which accounted for 51.5% of the total variation. The results also revealed that rice varieties were ordered into four distinct PCA clusters. Genotypes TOG 5681, FKR 19, WITA 4, NERICA-L38, and NERICA-L28 were quite distinct from other genotypes. Genotypes in clusters IV (4 and 45) flowered and reached physiological maturity earlier than other genotypes, while the variety NERICA-L38 was the tallest. Additionally, genotypes in clusters I and IX (1, 18, 22, 24, 25, 30, 35, 36, and 48) produced the highest number of tillers, and together with genotypes in cluster III (3, 14, 16, 21, 28, 38, 41, 43, and 47), the highest number of panicles. These genotypes in different clusters could be used in future hybridization programs. Hence, candidate future genotypes with good characteristics could be selected from these clusters (Akande *et al.*, 2007). Akande *et al.* (2007) further suggested that improved varieties, which are more productive than those currently grown by farmers, can be developed through mutation breeding, introduction, recombination, and selection. However, the use of morphological traits in germplasm classification has been met with difficulties, particularly in rice, as the technique is inefficient (Virk *et al.*, 2000). This could be due to environmental or climatic factors imposed on the genotypes, leading to differences in results based on morphological grouping, particularly when experiments are repeated in time and space (Morakinyo and Ajibade, 1998).

Ten polymorphic SSR primers used to screen the 48 rice genotypes in this study generated 49 alleles (average = 4.9 alleles per locus), indicating moderate diversity among rice genotypes. Differences in the number of alleles per locus could be due to the number of samples used, their genetic background, and most importantly, the nucleotide repeat in the SSR. Markers with PIC values ≥ 0.5 are considered highly informative for genetic studies and are extremely useful in determining the polymorphism of a marker at a specific locus (Sharma *et al.*, 2009). Markers, RM125, RM152, RM154, RM408, RM413, had PICs > 0.5 when evaluated among the genotypes studied. The identified markers' ability to resolve the level of heterozygosity within the studied varieties makes them a useful tool in future for rice genetic improvement programs. The mean PIC value observed in this study was close to and in conformity with the PIC values reported independently in previous studies on rice cultivars, landraces, and wild relatives (Ravi *et al.*, 2003; Ogunbayo *et al.*, 2005).

The presence of a two structured population similar to reports from Rashimi *et al.* (2017) and Mishra *et al.* (2019) but differed from the report of Yamasaki and Ideta (2013) who reported six subgroups within the Japanese rice population. However like the report of Ndjiondjop *et al.* (2018), the NERICAs created a separate peculiar subgroup revealing a clear population stratification between the intraspecific and interspecific rice genotypes. This peculiarity in stratification can be attributed to ecological adaptation of genotypes to ecologies. In addition, autogamous breeding system also plays a significant role in structuring the genetic variation within and among hierarchical groups or populations in rice varieties as opined by Ndjiondjop *et al.*, (2018).

The study further showed that diversity within the intraspecific and interspecific genotypes evaluated in this study was in proximity. Garris *et al.* (2005) had predicted a partitioning of diversity among rather than within populations in the absence of human-mediated gene flow between populations. More within population variation was observed than between population variation. An implication of main selective breeding activities to improve the rice germplasm. The narrow genetic base observed in this study have been predicted in previous studies probably due to the more severe domestication bottlenecks and breeding activities, constructing the genetic pool in order to improve and produce ago-ecologies' adapted rice varieties to meet up the increased production capacity (Reig-Valiente *et al.*, 2016)

Five main clusters generated from 48 rice genotypes using the UPGMA cluster analysis, based on genetic similarity, demonstrate the robustness and reliability of SSR data for the classification of rice genotypes into different heterotic groups, regardless of the genetic background, relationship, and location of intra- and interspecific lowland rice genotypes. Thus, SSR markers provide a reliable assessment of genetic diversity. Such fingerprinting makes the identification and characterization of genotypes efficient and will help in selecting the alleles of the recurrent parent in backcross progeny. According to Kumar *et al.* (2012), varietal profiling based on SSR markers is more reliable than with other markers since SSR markers can detect genetic variations at a greater resolution among closely related lines. Genotypes of similar origin or pedigree may have similar genes. Hence, crosses between such genotypes are generally not recommended since the resulting progeny may not show a genetic gain. As evident in the current study as well as in other studies (Torkpo *et al.*, 2006), information on genetic relatedness among different genotypes is useful not only for breeding purposes but also for germplasm conservation. This was evident in the current study, where cluster II contained the highest number of closely related rice genotypes, with a clear clustering of intraspecific varieties in subcluster IIA and interspecific genotypes on subcluster IIC and IID, a clear delineation between subcategories further showing the ability of the SSR markers to resolve within varietal differences. This was also evident in the other clustered groups in the study. The presence of checks and both parent genotypes clustering separately is an indication of their different genetic background.

Thus, SSR marker-based grouping was not consistent with the morpho-agronomic data depicting the influence of the environment on the morpho-agronomic traits of rice varieties evaluated in this study.

Conclusion

In this study, forty-eight rice genotypes were characterized using morphological and SSR molecular markers. The genotypes responded differently in the twelve environments considered in this research. The result also demonstrated the usefulness of AMMI and GGE biplot analyses in the interpretation of data from multi-environment experiment. The twelve sites used in this research work were observed as belonging to a single mega-environment where NERICA-L8, NERICA-L12, NERICA-L33, NERICA-L42 and NERICA-L56 were identified as the superior genotypes in terms of stability and yield. The implication of this finding is that, the same rice genotypes could be confidently deployed to these environments with optimal adaptation. The finding that all the twelve environments formed four mega-environment suggests that there is a need for further insight into the impact and magnitude of GEI in a larger environment with broader differences in climatic and ecological conditions. Moreover, heterosis can be maximize in the next breeding program when one of the genotypes in any cluster, especially in cluster 7 combines with TOG 5681 in cluster 1 based on SSR grouping. The molecular characterization was able to establish in concrete terms the extent of genetic relationship among the 48 genotypes studied. Thus, SSR fingerprints of the 10 primers emphasised the superiority of molecular marker in similarity grouping over and above morphological grouping. Overall, this study demonstrates that molecular markers, particularly SSR markers, are useful in establishing distinct relationships among genotypes, which could not have been revealed by morphological methods alone, especially since phenotypes are influenced by the environment (Torkpo et al., 2006).

Abbreviations

NERICA – New Rice for Africa

SSR – simple sequence repeat

ESTs – expressed sequence tags

PCA – Principal component analysis

PIC – Polymorphic information content

TOG- Tropical Oryza Glaberrima

MET- Multi- Environment trial

Declarations

Ethical Approval and Consent to Participate

Not Applicable

Consent for Publication:

Not Applicable

Availability of data and Materials:

The datasets supporting the conclusions of this article are provided within the article.

Competing Interests:

The Authors declare that they have no competing interests.

Funding:

Molecular work partly funded by IRRI, Los Banos, Philippines.

Authors' contributions

Conceptualization and Methodology: AO, MS., GBG, and DKO; Investigation and original draft preparation: AO., MS, and GBG; Critical review and editing: AO., GBG., DKO., MS., KAS., STA., AS., MGA, NAA, JAA, ODA, MAA, SO and OA. All authors then reviewed and approved the paper before publication.

Acknowledgements:

The authors are deeply grateful to the Staff of Lowland Breeding Unit, AfricaRice in Benin and Nigeria for their assistance during the fieldwork. We are grateful for the in-depth analysis carried out by Mr. Peter Oyelakin, IITA, Nigeria; Ms. Andriette Ferreira of AUDA-NEPAD helped with the location map.

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Figures

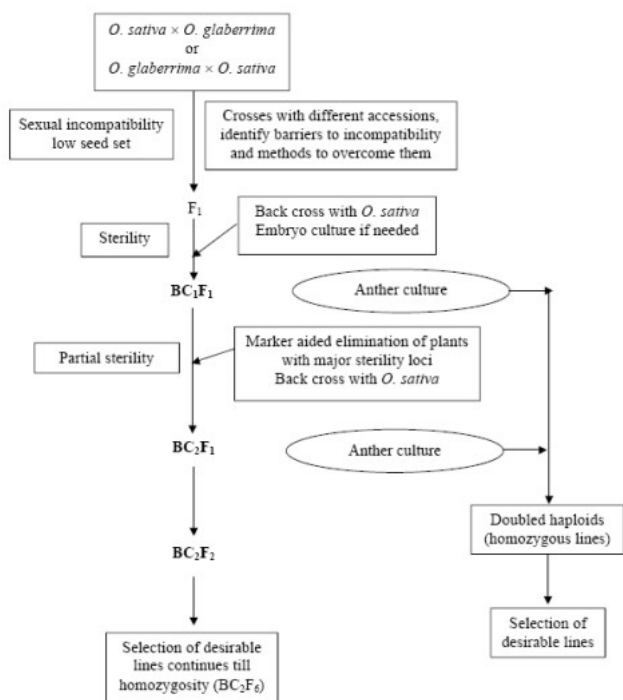


Figure 1

Proposed scheme to obtain fertile lines from interspecific crosses between *O. sativa* and *O. glaberrima* (Sarla and Swamy2005).



Figure 2

Sketched map indicating the three locations used for the multilocational trials

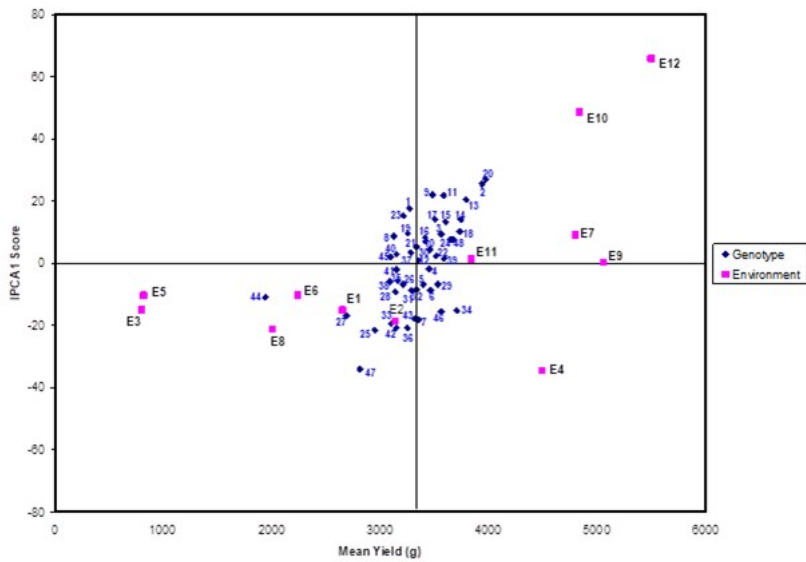


Figure 3

biplot of AMMI for 48 rice genotypes in 12 environments.

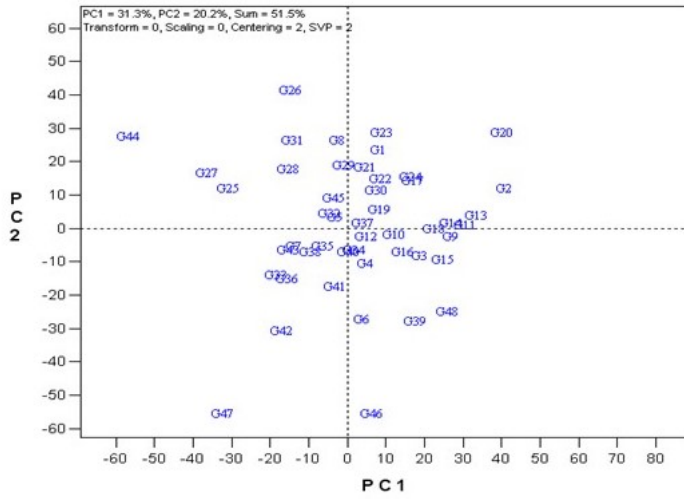


Figure 4

Plot of PC1 and PC2 showing the relationship between clusters of 48 rice accessions in wet seasons of 2008 and 2009 across locations.

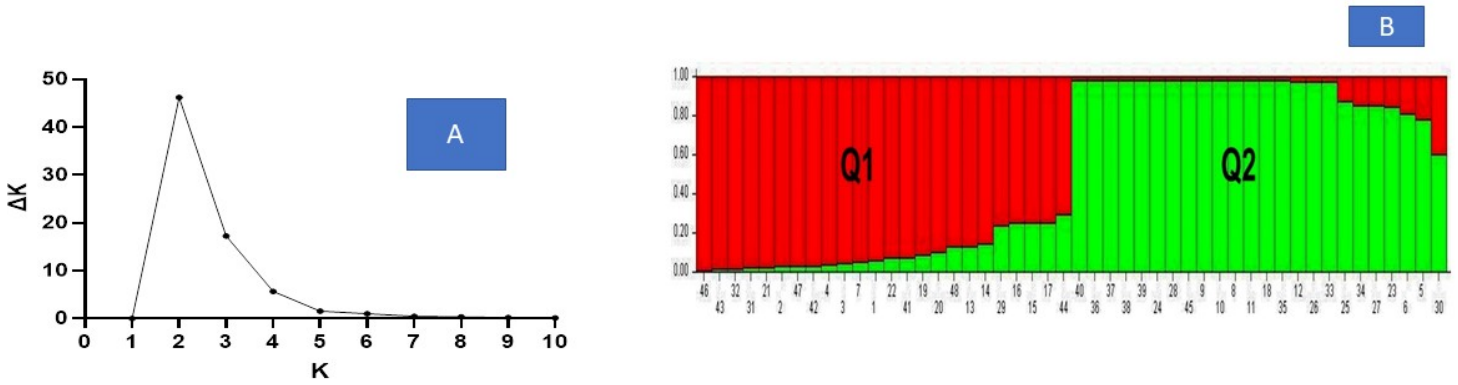


Figure 5

a. Plot of ΔK against K ranging from 1 to 10 from 10 iterations, b. Population structure among rice genotypes evaluated

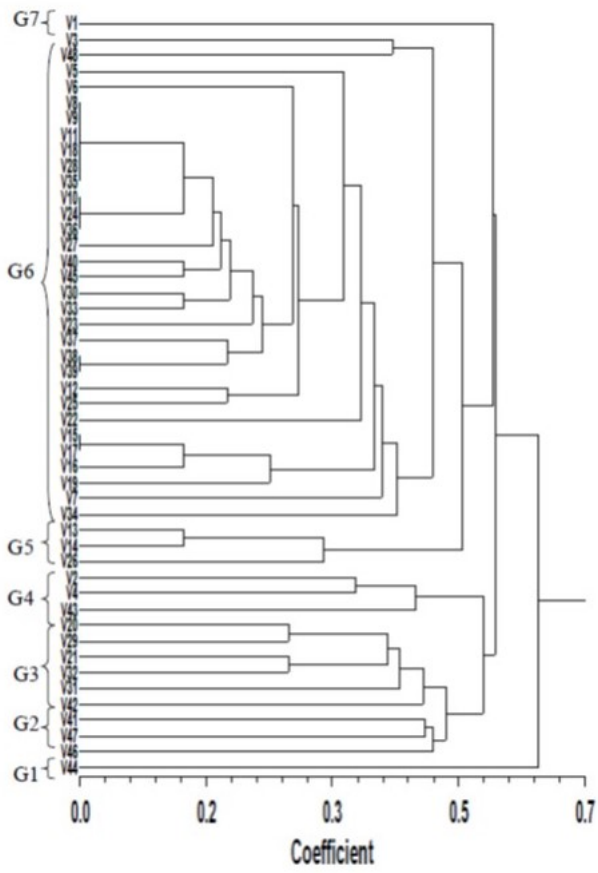


Figure 6

Dendrogram of 48 rice accessions derived from unweighted pair group mathematic average (UPGMA) based on Jaccard's coefficient with 10 SSR markers.