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Agro-Morphological Characterization and Assessment of Variability within a Germplasm of Benin Rice (*Oryza sativa* L.) Varieties

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Abstract

The knowledge of the agro-morphological diversity within a crop and its distribution across agro-ecological zones could be of a great help in the management of its germplasm and the development of strategies for its improvement. In this study the phenotypic diversity within a germplasm of 127 accessions of Benin rice varieties is assessed through agro-morphological characterisation with 24 qualitative and 20 quantitative variables. Within the quantitative parameters, the most variable were plant height (PlH), leaf length (LeafL), panicle leaf length (FlagLL), number of tillers (NberTillers), yield (GrYield), sowing to heading cycle (SHC), sowing-to-maturity cycle (80%SMC), leaf width (LeafW), while the less variable were grain length, grain width, panicle length. Within the qualitative ones, form of the caryopses, attitude of ramifications and color of apex (1) expressed the higher variability. A large phenotypic diversity as measured by the Shannon-Weaver diversity index, was observed within accessions (H= 0.72) and across agro-ecological zones (0.29) and phenotypic groups (0.48). Lowland accessions being less diverse than those cultivated in highland. With the country, Zone IV including Boukoumbé, Djougou, Kobli, Matéri, Sinendé and Tanguiéta is the main area of high diversity. Multivariate analysis classified accessions into three groups (Gp1, Gp2 and Gp3) of different characteristics.

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Introduction

Rice (*Oryza spp.*) is an economically important crop in the world (FAO, 2016). It belongs to the Oryzae tribe and the Poaceae family. Among the 23 species comprising the genus *Oryza*, only two species (*Oryza sativa* L., Asian rice and *Oryza glaberrima* Steud, African rice) are grown (Vaughan et al., 2003). Asian rice (*Oryza sativa* L.), comprises two main sub - species which are *indica* (adapted to floating tropical and subtropical regions or lowland and irrigated agricultural systems) and *japonica* (adapted to tropical highlands) (Tendro Radanielina et al., 2013). Several collections of these species (*Oryza sativa* L., and *Oryza glaberrima* Steud) exist throughout the world (Barry et al., 2007) and mostly at the genebanks of IRD in France, The

International Institute of Tropical Agriculture in Nigeria, The International Rice Research Institute in Philippines and the Japan Institute of Genetics in Japan (Sow et al., 2013). Contrarily to Guinea, Mali, Nigeria and Niger, that have their rice varieties well collected, studied and stored in different gene banks (Nuijten et al., 2009; Sow et al., 2013) collection from Benin is poorly represented. However, the country has a potential of 205 000 ha of lowlands, plus more than 110 000 ha of irrigable lands (MAEP, 2011) among which barely 10% is currently being used for rice production (MAEP, 2011). Benin has an old tradition of rice cultivation with, for instance, about 234145 tons in 2015 (FAO, 2016).

Due to the importance of rice as one of the major world food crops, its genetic diversity has attracted great interest of researchers (Roy et al., 2016; Sinha et al., 2016). Morphological evaluation is a preliminary step to estimate the variability and relationship among cultivars (Smith et al., 1991). To establish a country specific successful rice improvement programme, it is very much essential to discover the morphological variability of existing varieties within the country. It is fundamental in order to provide information for plant breeding programs (Ahmed et al., 2016). Local varieties are valuable as they possess a huge treasure of genetic material for development and improvement programs. Several researchers reported the use of agro-morphological markers in the characterization and study of rice (*Oryza sativa* L.) germplasm diversity in China (Yawen et al., 2003), India (Roy et al., 2013), Vietnam (Nguyen et al., 2009), Nepal (Mahendra et al., 2013) and even in Africa (Semon et al., 2005; Barry et al., 2007; Nuijten et al., 2009; Sow et al., 2013). In Benin and in our knowledge, no genetic diversity study on rice is reported. We report in this paper, a study conducted to assess the diversity within a national collection of rice (*Oryza sativa*) using agromorphological characters to provide useful information for conservation and breeding programs.

Materials and methods

Plant material, cultivation method and data collection

A total of 136 accessions (Table 1) including 95 accessions collected from different villages and regions of Benin (Odjo et al., 2017a), 32 'unique' off-types and nine check varieties obtained from AfricaRice genebank in Cotonou were considered. The check varieties included three lowland *indica* (IR841, Adny11, WAB 32), one upland *japonica* (Gambiaka Kokou), one African rice (CG14) and four Asian-African interspecific hybrids (Nerica-L-20 Nerica-L-19, Nerica 4 and Nerica 2) (Table 1).

Clean seeds of all the accessions were sown on a nursery and 21 days after emergence, the most vigorous seedlings of each genotype were transplanted to the field, following the procedure reported by Nascimento et al. (2011). The experiment was conducted at the experimental rice production lowland based at laïnta-Cogbé in the district of Covè in southern Benin during the rainy season of 2014 and of 2015. The field was watered by irrigation during dry periods. The experiment design was Alpha lattice with three replicates. Each replicate consisted of 14 blocks comprising 14 plots each. Each plot includes 5 lines of 24 plants spaced 20 cm apart, with the rows spaced 0.50 m apart. The plot size for each accession was 1m ^{2} (1m × 1m). A total of 200 kg/ha Nitrogen-Phosphorous-Potassium (NPK) (15:15:15) was applied to all plots just after thinning. Urea was also applied 45 days after sowing at a rate of 100 kg/ha. Regular weeding was done when necessary.

Table 1. List of rice (*Oriza sativa* L.) accessions used for the agro-morphological characterization and their origins.

Région	District	CS	NA	Accession names	Field code	AZ
Central	Bantè	Adjantè	1	Gambiaka (4)	V14	Zone V
		Atokolibé	1	Gambiaka (3)	V13	Zone V
	Glazoué	Sowé	3	Olotchoumédji (1); Olotchoumédji (2); Olotchoumédji (3)	V61; V113; V126	Zone V
	Savalou	Tchetti	2	IR 841 (1); Jasmine	V32; V34	Zone V
	Savè	Gobé	1	Gambiaka (2)	V12	Zone V
North	Banikoara	Founougo	6	ADNY11; Béris 21; Unknown (2); L 19; Burkina Faso; Unknown (24)	V1; V3; V22; V38; V112; V121	Zone II
		Goumonri	2	De Gaulle (3); Orou Mississi	V10; V62	Zone II

Région	District	CS	NA	Accession names	Field code	AZ
	Bassila	Allan	3	Unknown (7); Unknown (11); Nérica-L-20	V27; V31; V60	Zone V
		Kodowari	1	Lobolobo (2)	V41	Zone V
	Boukoumbé	Dikoumini	5	Mountain rice (1); BL 19; Mountain rice (2); Pouessoir; Imouri Doki	V91; V92; V93; V94; V95	Zone IV
		Dipokor 1	6	Gambiaka (6); Imon Boka; Namonhan Tadana; Projet; Imon Pia; Issomonhan	V96; V97; V98; V99; V100; V101	Zone IV
	Copargo	Kpassabéga	3	Kri Ita; Lèbèlèbè; Mri mouri	V36; V39; V102	Zone IV
	Djougou	Dendougou	3	Tchroéitama; Tchroinès; Tchroinesma	V74; V75; V76	Zone IV
	Gogounou	Ouèrè or wèrè	2	Yayi Boni; Guibitounga	V79; V103	Zone II
	Kandi	Sam	5	Inaris; Orou-Kpété; Yayi Boni Kpika; Carder souan; Unknown (19)	V20; V63; V80; V115; V116	Zone II
		Sinawongourou	6	Batoun mongni; Unknown (6); Unknown (9); Guibitounga; Unknown (16); Unknown (25)	V2; V26; V29; V108; V109; V122	Zone II
	Kobli	Pégou	4	Koumouloumonga; Gambiaka (5); Koumouloukonga; Nadréki	V87; V88; V89; V90; V44	Zone IV
	Malanville	Madécali	6	Gazéré; Grand Djimbo; Unknown (10); Koitéguizé; Petit Djimbo; R8	V15; V16; V30; V35; V64; V67	Zone I
	Matéri	Kondo	4	Mongni Koukourikê; Mongni Pam pam; Gambiaka laguê; Mongni Poe Lakê	V83; V84; V85; V86	Zone IV
		Toubougnini	3	WAB 32; Nérica 2; Mongni douab lack	V78; V81; V82	Zone IV
	Nikki	Koni	4	Carder (3); Unknown (3); Unknown (5); Unknown (22)	V6; V23; V25; V119	Zone III
		Sérékali	3	Carder (2); IR 841 (2); Mayada	V5; V33; V42	Zone III
		Totorou	2	Précoce; Tissu	V66; V77	Zone III
	Ségbanna	Lougou	3	Unknown (4); Moepian; Moepoua (2)	V24; V43; V45	Zone II
		piami	1	Moepoua (1)	V44	Zone II
	Sinendé	Fô-bouré	3	De Gaulle (2); Monri Bosserou; Ponnin;	V9; V52; V65	Zone III
		Fô-Tancé	3	Mouri Kplinnou; Mouri Souri Inè; Mouri Souri Ita	V54; V55; V56	Zone III
		Sèkèrè	4	Mongni gbénou (1); Mongni gbénou (2); Mongni Kpika; Unknown (21)	V46; V47; V48; V118	Zone III
		Sèmèrè	2	Lobolobo (1); Unknown (13)	V40; V105	Zone IV
	Tanguiéta	N'dahonta	6	Monniti (1); Monniti (2); NéricaX (1); Tchewèrèga; Monniti (3); NéricaX (2)	V50; V51; V57; V72; V124; V125	Zone IV
	Tchaourou	Bétérou	3	Carder (1); Monwassou; Unknown (14)	V4; V53; V106	Zone V
		Kaki Koka	1	Unknown (20)	V117	Zone V
South	Comè	Gativé	1	L 20	V37	Area VIII
	Covè	Koussin-lélé	7	Unknown (1); Nérica-L-20; Tchinhin; Unknown (12); Unknown (17); Unknown (18); Unknown (26)	V21; V59; V73; V104; V110; V111; V127	Zone VI

Région	District	CS	NA	Accession names	Field code	AZ
	Dangbo	Hêtin- Sota	1	Unknown (23)	V120	Zone VIII
	Djidja	Madjavi		De Gaulle (1); Souhaton (1); Souhaton (2); Souhaton (3); Souhatôn (4); Souhannin	V8; V68; V69; V70; V71; V114	Zone V
	Dogbo	Dévé	1	Nérica 14	V58	Zone VI
	Houéyogbé	Houinga-houégbé	1	Unknown (15)	V107	Zone VI
	Kétou	Dogo	3	Imonhan Ipèyi; Imonhan Iwonnin; Unknown (8)	V18; V19; V28	Zone V
	Lalo	Tandji	5	Monlou takpitô; Chamarica (1); Gambiaka (1); Imondobi; Chamarica (2)	V49; V7; V11; V17; V123	Zone VII
	IITA	AfricaRice	9	Gambiaka Kokou (T1); Nerica-L-20 (T2); Nerica-L-19 (T3); ADNY11 (T4); WAB 32 (T5); Nerica 4 (T6); Nerica 2 (T7); IR 841 (T8); CG14 (T9)	T1; T2; T3; T4; T5; T6; T7; T8; T9	

NA = Number of accessions CS= Collecting site AZ = Agro-ecological zone.

The accessions were characterized according to the descriptors established by the International Rice Research Institute and Bioversity International (Bioversity International, 2007). A total of 20 quantitative (Table 2) and 24 qualitative descriptors (Table 3) were used. Data were recorded on five to ten plants of the inner row from the seedling stage to harvest. For each accession, panicles of five well identified plants from the inner row were individually harvested while the remaining ten were bulk-harvested.

Table 2. List of quantitative variables.

Phase	Variables	Symbols	Measurement
Reproductive	Sowing to heading cycle	SHC	Date at which 80% of the plants spied.
	Ligule length	Lig_Leng (mm)	From the base of the collar to the top of the leaf ligule below the panicle leaf.
	Leaf length	Leaf_L (cm)	From the ligule to the top of the leaf blade (leaf beneath the panicle leaf)
	Leaf width	Leaf_W (cm)	Width of the widest part of the leaf below the panicle leaf
	Flag leaf length	Flag_LL (cm)	From the ligule to the summit of the leaf blade
	Flag leaf width	Flag_LW (cm)	Width of the widest part of the panicle leaf
Maturity (pasty grain stage)	Stem Diameter (mm)	Diam_Stem (cm)	Outer diameter at the basal internode of the main tile
	Plant height (cm)	Pl_H (cm)	From the surface of the soil to the top of the longest panicle
	Number of tillers	Nber_Tillers	Counting tillers with panicles and those without panicles
Maturity (hard grain stage)	Sowing-to-maturity cycle	80% SMC	Date that 80% of the grains on a panicle are completely walls
	Number of panicles per plant	Nber_Pan	number of panicles on an area of one square meter before harvest
	Number of grain per panicle	Nber_gr_pan	total grain count per panicle
	Number of full grain per panicle	Nber_FG_pan	Number of well-Developed and fully filled grains.
	Number of primary branching	Nber_Prima_Bran	Count the number of primary ramifications
	Number of secondary branching	Nber_Secon_Bran	Count the number of secondary branches
	Panicle length	Pan_L (cm)	Length of the main axis of the base (node at the base) at the top
	Grain yield (g/m^2)	Gr_Yield	Yield in g / m^2 at 14% of the moisture content
Post-harvest	Grain length	Gr_L (mm)	From the base of the glume to the top of the apex, excluding the aristation
	Grain width	Gr_W (mm)	Greater width of the lemma and palm with a calliper
	1000 grain weight	Gr_Wei	Mean weigh of 3 samples of 1000 grains with a water content of 13%.

Table 3. List of qualitative variables.

Phase	Variables	Symbols	Code
Vegetative (seedling stage)	Plant vigor	Pl_Vig	(1) extra vigorous, (3) vigorous, (5) normal, (7) weak
Vegetative (elongation stage)	Color of the base of the plants	CBP	(1) green, (2) purple lines, (3) light purple, (4) purple
	Anthocyanin coloration of the leaf sheath	CALS	(0) absent, (1) very weak, (3) weak, (5) medium, (7) strong.
	Leaf blade coloring	LBlC	(0) absent, (3) light green, (5) medium green, (7) dark green
	Distribution of anthocyanin / leaf blade	DALBl	(0) absent, (1) At the end only, (3) spotted
	Pubescence of the limb surface	Pubs_LSur	(1) glabrous or smooth, (2) intermediate, (3) pubescent
	Color of auricle	CA	(0) absent, (1) whitish, (2) yellowish green, (3) purple, (4) light purple
	Color of necklace	CN	(1) green, (2) light green, (3) violet
	Attitude of leaf blade	ALBl	(1) erect, (5) horizontal, (7) descending
Reproductive	Shape of the ligule	SL	(1) truncated, (2) pointed, (3) bifide
	Color of stigma	Col_Stig	(1) white, (2) light green, (3) yellow, (5) purple
	Attitude of the panicle leaf	APL	(1) erect, (3) semi-erect, (5) horizontal, (7) descending
	Angle of inclination of the rod	Ang_IRod	(1) erect, (3) semi-erect, (5) open
Maturity (pasty grain stage)	Anthocyanin coloration level of knots	ACKn	(0) absent, (1) purple, (3) purple lines
	Color Lemma and palea (1)	CLPa1	(1) White (3) gold and furrows in gold (4) brown (tawny) (5) brown spots on the green (8) Green (10) Purple (13) purple spots on the green
	Color of apex (1)	CAp1	(1) white (2) straw (3) brown (fawn) (4) green (5) red (6) red apex (7) purple (8) purple apex
	Panicle Exsertion	Pan_Exs	(3) partial exsertion, (5) fair exsertion, (7) good exsertion, (9) very good exsertion
	Attitude of ramifications	ARam	(1) compact, (3) semi-compact, (5) open, (7) horizontal,
Maturity (hard grain stage)	Color lemma and palea (2)	Col_LemPa2	7= purple 8= of the reddish to the purple clear 10=furrows purple 11= black
	Shelling	Shel	(3) Weak, (5) Moderate, (7) High, (9) Very high
	Shape of apex	Shap_Ap	(1) Sharp, (2) Curved
	Color of sterile lemma	Col_St Lem	(1) Straw, (2) Gold, (3) Red, (4) Purple
Post-harvest	Form of the caryopse	FoCary	(1) round, (2) semi-round, (3) half-spindle shape, (4) spindle shape
	Color of pericarp	Col_peri	(1) White, (2) Light brown, (4) Brown, (5) Red, (6) Variable purple

Data analysis

For each accession, and each variable observed, the mean of the observations on the 5 individual plants from each of the two trials were calculated. These means were subjected to analysis of variance (ANOVA) with the *XL-STAT 2016* software to test the effect of the sub-blocks and the year. In the absence of significant sub-block and year effects an adjusted overall mean was calculated for each accession and each variable, and were subsequently used to analyse agro-morphological diversity.

For an efficient statistical analysis, a correlation tests were performed on the 44 variables. Thus, a Chi-2 independence test was performed on the 24 qualitative variables using R software and 14 most discriminating variables at 0.45 of correlation was selected. Similarly, a Pearson correlation test was performed on the 20 quantitative variables with the R software to define variables (14 in total) that contributed most to the discrimination of accessions of rice in Benin.

Principal component analysis (PCA) was performed on the standardized quantitative data for 14 traits, followed by the assignment of the different accessions into groups by an agglomerative hierarchical clustering (ACH) method and Factor Analysis (FA) with XLSTAT 2016 software. Dissimilarities were computed based on the Euclidean distance and aggregation of accessions was based on the Ward method (Ward, 1963). The diversity of phenotypic traits was estimated using the Shannon–Weaver diversity index (H) (Shannon and Weaver,

1948) computed with Microsoft Excel 2010 software and the 28 most variable characters (14 quantitative and 14 qualitative) following Abdi et al. (2002); Sanni et al. (2008).

Results

Variability of the qualitative characters

The majority of Benin's rice varieties (68.38%) show good strength. Only 31.62% are less vigorous. Among the most vigorous there are Gazéré from Madécali and Mri monri from Kpassabega (Fig. 1b). The great phenotypic variability shows that 51.47% of the rice varieties of Benin exhibit better panicle exsertion. It is followed by good and very good. Partial exsertion represents only 1.47% of the varieties (Fig. 1a). For a good panicle exsertion varieties such as Gambiaka from Adjantè and Koumouloukonga from Pégou are indicated. At the outlet, the grains of the rice varieties have varied colors. Despite this, the color ranging from reddish to purple clear (66.91%) is the most represented. It is followed by grains with furrows purple (27.94%) (Fig. 1c). There is also variability in the pericarp levels observed in the collection. The majority of the varieties have a light brown color (92.65%) (Fig. 1e). Most varieties are rapidly shelling. Only 0.74% are slightly weak (Fig. 1d). The variability of the most discriminative qualitative variables of the rice varieties cultivated in Benin is presented in the Table 4.

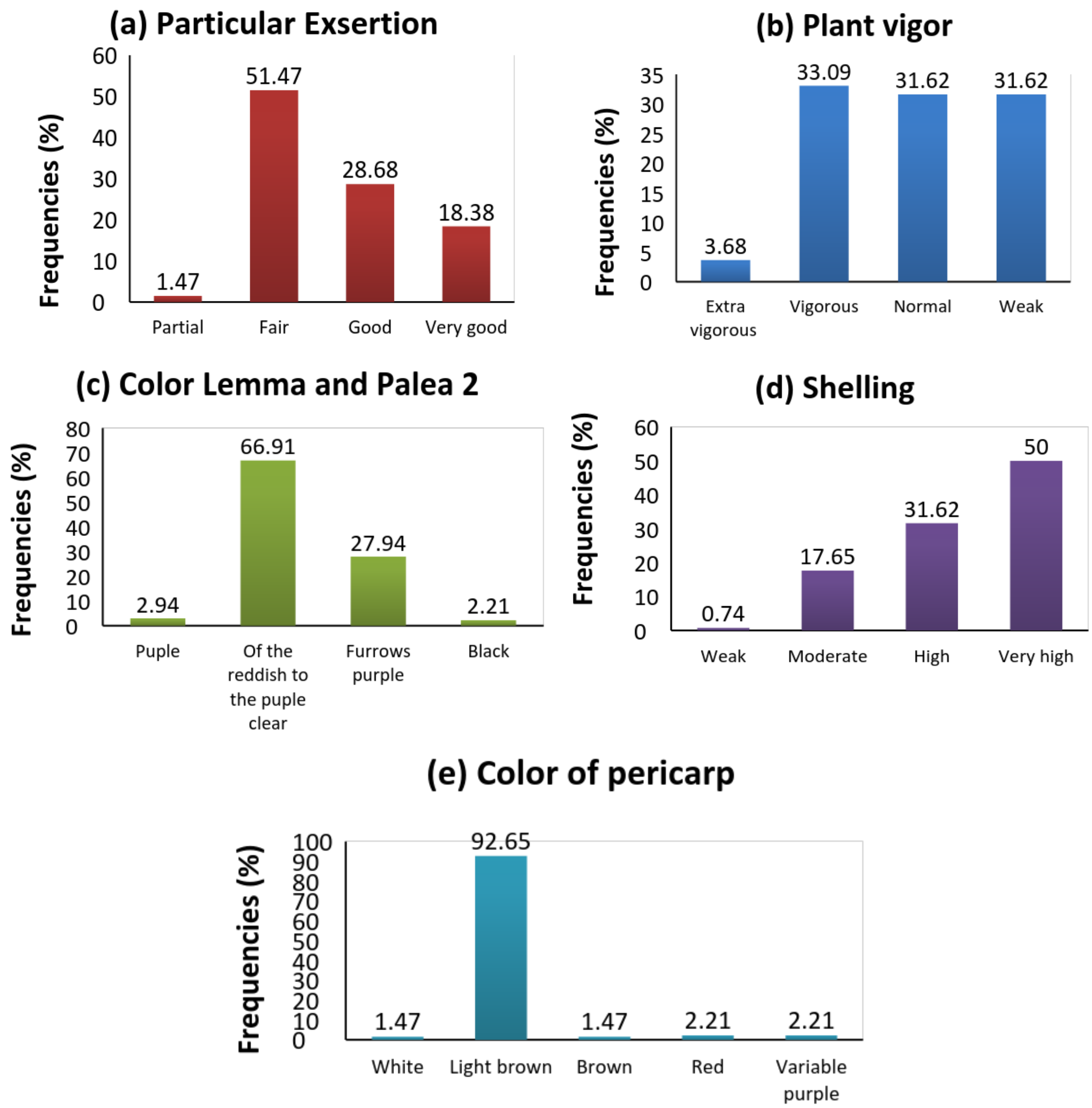

Fig. 1: Variability of less discriminant qualitative characters of Benin rice varieties.

Table 4. Variability of 14 most discriminant qualitative characters of Benin rice varieties.

No.	Qualitative variables	Symbols	Percentage parameter (%)
1	Color of the base of the plants	CBP	(81.62%) green, (8.09%) purple lines, (3.68%) light purple, (6.62%) purple.
2	Anthocyanin coloration of the leaf sheath	CALS	(81.62%) absent, (3.68%) weak, (8.09%) medium, (6.62%) strong.
3	Anthocyanin coloration of the Leaf blade	LBlC	(83.09%) absent, (16.91%) Present
4	Distribution of anthocyanin / leaf blade	DALBl	(80.88%) absent, (9.56%) At the end only, (9.56%) spotted.
5	Color of auricle	CA	(11.76%) absent, (72.29%) whitish, (8.82%) yellowish green, (5.15%) purple, (1.47%) light purple.
6	Color of necklace	CN	(10.29%) green, (81.62%) light green, (5.09%) purple.
7	Attitude of leaf blade	ALBl	(96.32%) erect, (2.94%) horizontal, (0.74%) descending.
8	Shape of the ligule	SL	(18.38%) truncated, (0.74%) pointed, (80.88%) bifide.
9	Attitude of the panicle leaf	APL	(65.44%) erect, (33.09%) semi-erect, (0.74%) horizontal, (0.74%) descending.
10	Anthocyanin coloration level of knots	ACKn	(86.76%) absent, (12.50%) purple, (0.74%) purple lines.
11	Color Lemma and palea (1)	CLPa1	(51.47%) White (0.74%) gold and furrows in gold (3.68%) brown (tawny) (29.41%) brown spots on the green (13.24%) Green (0.74%) Purple (0.74%) purple spots on the green
12	Color of apex (1)	CAp1	(18.38%) white (0.74%) straw (1.47%) brown (fawn) (55.15%) green (8.82%) red (0.74%) red apex (8.82%) purple (5.88) purple apex
13	Attitude of ramifications	ARam	(5.88%) compact, (52.94%) semi-compact, (32.35%) open, (3.68%) horizontal, (5.15% Fall
14	Form of the caryopse	FoCary	(8.82%) round, (32.35%) semi-round, (4.41%) half-spindle shape, (35.29%) spindle shape, (19.12%) long-spindle shape.

The Chi-square independence test performed on the 24 qualitative variables with the R software reveals that the 14 variables in Table 5 are the most related and discriminating of the different varieties of rice grown in Benin. This table presents the correlations between the 14 most discriminant variables chosen for the rest of the analysis. From this table it is noted that certain variables have no dependence on each other such as the color of the auricle and the attitude of the branches. However, the color of the base of the plants is strongly related to the anthocyanin coloration of the leaf sheath, the leaf blade staining, the distribution of the anthocyanin at the leaf blade, The color of the collar, the attitude of the leaf blade, the attitude of the panicle leaf, the anthocyanin coloration at the knots, the color Lemma and palea (1), the color of the apex (1), and the shape of the caryopse. But it is moderately related to the shape of the ligule and weakly related to the attitude of the ramifications.

Variability of the quantitative characters

Table 6 shows the minimum, maximum, mean, standard deviation and coefficient of variation of the quantitative variables used. A great variability is observed in all traits except for the leaf width, grain length and weight of the grains. The potential yield oscillates between 3312.1 and 31214.3 Kg/ha with an average of 13823.7 Kg/ha. Similarly, yield components such as the number of panicles per plant, the number of tillers per plant and the weight of one thousand grains exhibit great variability. Weight of one thousand grains for instance varies from 20.66 to 37.54 g and the number of panicles per plant varies from 5 to 27. Despite this great variability, the rice varieties of Benin are very productive.

Analysis of variance (ANOVA) performed on the 20 quantitative variables (Table 7) measured indicated a high phenotypic variability (*p*-value<0.0001) at the threshold of 0.05. No significant effect of the blocks was noted. For the variation of the traits measured from one year to another, there is a significant effect of the year on the characters such as the sowing cycle, the seed maturity cycle, the length of the leaf, the width of the leaves, the width of panicle leaves, stem diameter, number of tillers, number of panicles, number of primary and secondary branches, length of panicles, grain width and yield. Thus, for all characters, the adjusted values will be used in the rest of the study. There is little influence of the year on accessions.

Table 5. Matrix dependence between the most discriminating qualitative variables.

Variables	CBP	CALS	LBlC	DALBl	CA	CN	ALBl	SL	APL	ACKn	CLPa1	CAp1	ARam
CALS	276***												
LBlC	124***	128***											
DALBl	111***	109***	109***										
CA	80***	86***	50***	81***									
CN	57***	57***	39***	52***	86***								
ALBl	36***	39***	13**	29***	33*	62***							
SL	23**	18*	8**	15*	37***	48***	95***						
APL	55***	61***	28**	50***	44*	11^{ns}	154***	3^{ns}					
ACKn	93***	94***	65**	62***	51***	34***	12^{ns}	16*	7^{ns}				
CLPa1	46***	50***	46**	44***	43*	16^{ns}	53***	10^{ns}	48*	25*			
CAp1	129***	125***	90**	119***	65***	47***	42*	17^{ns}	73***	75***	75**		
ARam	25*	26*	18**	42***	21^{ns}	12^{ns}	33*	20^{ns}	47***	16*	52***	41^{ns}	
FoCary	35***	35***	23**	45***	34*	32***	15^{ns}	8^{ns}	27^{ns}	19*	20^{ns}	52**	34**

Table 6. Basic descriptive statistics of quantitative variables.

Statistical	Minimum	Maximum	Average	Standard deviation	Coefficient of variation
SHC	61.67	133.17	96.75	19.17	19.82
80% SMC	91.00	161.17	128.78	19.12	14.84
Diam_Stem	0.35	0.66	0.50	0.06	11.91
Pl_H	82.76	219.80	141.24	28.61	20.26
Leaf_W	0.85	2.67	1.40	0.22	15.65
Flag_LW	1.12	2.43	1.60	0.22	14.07
Gr_W	2.54	3.62	2.95	0.21	6.95
Leaf_L	25.39	84.88	53.00	13.77	25.97
Flag_LL	21.13	83.13	44.01	11.62	26.41
Gr_L	7.40	10.64	9.48	0.65	6.85
Lig_Leng	0.37	3.95	2.08	0.67	32.15
Pan_L	20.08	42.92	27.84	3.21	11.55
Nber_FG_pan	60.85	221.26	133.86	30.63	22.88
Nber_gr_pan	65.30	231.34	145.20	31.83	21.92
Nber_Pan	5.61	27.77	13.97	4.40	31.48
Nber_Prima_Bran	8.43	29.18	13.22	2.47	18.68
Nber_Secon_Bran	6.57	60.37	32.22	10.92	33.90
Nber_Tillers	7.34	53.59	21.13	6.94	32.82
Gr_Wei	20.66	37.54	27.00	2.47	9.14
Gr_Yield	331.21	3121.43	1382.37	581.63	42.07

The Pearson correlation test performed on the 20 quantitative variables with the R software revealed that 14 variables (Table 8) are the most related and discriminating parameters. These variables will be used for the rest of the analysis. All significant correlations observed between the variables are positive except that between the width of the panicle leaves and the length of the ligule which is negative (Table 8). Positive correlations include those that are highly significant (R = 0.99, *p* = 0.0001), between yield and number of panicles per plant (r = 0.81, *p* = 0.0001), between the number of seeds per panicle and the number of full seeds per panicle (r = 0.99, *p* = 0.0001), between plant height and variables such as leaf length and the length of the panicle leaves (respectively r = 0.86, r = 76, *p* = 0.0001) and between the length of the leaves and the length of the panicle leaves (r = 0.74, *p* = 0.0001). In addition, there was also a slight correlation between the number of panicles per plant and the number of tillers (r = 0.60, p = 0.0001), between yield and variables such as sowing, The number of seedlings per panicle, the number of seeds per panicle, the height of the plants, the length of the leaves and the length of the panicle leaves, between the height of the plants and the variables such as sowing cycle, seedling maturity cycle, number of grains per panicle, number of full grains per panicle, stem diameter and length of the ligule, between sowing cycle and variables such as number of grains by panicle, the number of full leaves per panicle, the diameter of the stems, the length of the leaves and the length of the panicle leaves, between the seedling maturity cycle and the same variables as the seedling sowing cycle, between the number of seeds per panicle and the length of the panicle leaves, the diameter of the stems and variables such as the length of the leaves and the length of the panicle leaves, between the width of the leaves and the length of the panicle leaves, between the length of the leaves and the length of the ligule.

Table 7. Effect of accession, repetition (R), block, year and interaction (Year * Accession).

Variables	Source	Model	Accession	Reply	Block	Year	Year * Accession
	DDL	280	135	2	7	1	135
SHC	MC	1082.53	2184.5	139.64	7.52	724.71	15.03
	F	79.24 ***	159.9 ***	10.22 ***	0.55 ns	53.05 ***	1.1 ns
80% SMC	MC	1077.26	2173.17	142.77	14.3	1158.3	13.78
	F	77.6 ***	156.54 ***	10.28 ***	1.03 ns	83.44 ***	0.99 ns
Lig_Leng	MC	1.34	2.66	2.56	0.03	0.33	0.04
	F	45.63 ***	90.52 ***	87.07 ***	0.91 ns	11.24 **	1.26 *
Leaf_L	MC	552.78	1119.93	14.08	5.56	0.39	8.55
	F	70.13 ***	142.09 ***	1.79 ns	0.71 ns	0.05 ns	1.08 ns
Leaf_W	MC	0.16	0.29	1.87	0.01	1.4	0.01
	F	12.49 ***	21.62 ***	141.82 ***	0.91 ns	106.08 ***	1 ns
Flag_LL	MC	395.85.	787.04	5.44	2.79	1.19	6.56
	F	59.38 ***	118.07 ***	0.82 ns	0.42 ns	0.18 ns	0.98 ns
Flag_LW	MC	0.17	0.3	2	0.01	0.53	0.02
	F	11.48 ***	20.21 ***	132.97 ***	0.44 ns	34.98 ***	1.28 *
Diam_Stem	MC	0.17	0.3	2	0.01	0.53	0.02
	F	11.48 ***	20.21 ***	132.97 ***	0.44 ns	34.98 ***	1.28 *
Pl_H	MC	2399.29	4818.2	9.04	11.6	37.02	28.38
	F	122.95 ***	246.91 ***	0.46 ns	0.59 ns	1.9 ns	1.45 **
Nber_Tillers	MC	142.74	285.92	64.27	1.67	283	2.16
	F	92.55 ***	185.38 ***	41.67 ***	1.08 ns	183.48 ***	1.4 **
Nber_Pan	MC	61.1	114.8	265	2.87	301.98	3.4
	F	40.13 ***	75.4 ***	174.07 ***	1.89 ns	198.36 ***	2.24 ***
Nber_gr_pan	MC	3098.89	5770.41	924.43	330,49	240.39	270.98
	F	6.12 ***	11.39 ***	1.82 ns	0.65 ns	0.47 ns	0.53 ns
Nber_FG_pan	MC	2887.2	5339.87	2028.2	369.98	17.5	268.91
	F	5.77 ***	10.67 ***	4.05 *	0.74 ns	0.03 ns	0.54 ns
Nber_Prima_Bran	MC	23.02	36.14	132.8	1.32	637.06	4.09
	F	11.43 ***	17.94 ***	65.91 ***	0.66 ns	316.19 ***	2.03 ***
Nber_Secon_Bran	MC	355.25	704.8	87.08	12.26	432.98	10.33
	F	49.8 ***	98.81 ***	12.21 ***	1.72 ns	60.7 ***	1.45 **
Pan_L	MC	33.98	62.01	72.59	4.73	395.46	3.86
	F	8.18 ***	14.92 ***	17.46 ***	1.14 ns	95.14 ***	0.93 ns
Gr_L	MC	1.25	2.53	2.58	0	0	0.01
	F	216.21 ***	436.97 ***	447.18 ***	0.6 ns	0.31 ns	1.1 ns
Gr_W	MC	0.15	0.25	2.79	0	0.09	0.01
	F	27.43 ***	47.38 ***	523.05 ***	0.4 ns	15.98 ***	1.15 ns
Gr_Wei	MC	17.8	36.07	3.53	0	0	0.01
	F	4755.54 ***	9638.3 ***	942.93 ***	1.3 ns	0.82 ns	1.35 *
Gr_Yield	MC	1048265.2	1986864.7	2950828	66660.31	3131890	59021.84
	F	16.52 ***	31.31 ***	46.5 ***	1.05 ns	49.35 ***	0.93 ns

Table 8. Dependency Matrix among the most discriminating quantitative variables.

Variables	SHC	80% SMC	Nber_ Tillers	Nber_Pan	Gr_ Yield	Diam_ Stem	Pl_H	Leaf_W	Flag_ LW	Leaf_L	Flag_LL
SHC	**1**										
80% SMC	**0.99 *****	**1**									
Nber_Tillers	**0.20 ***	**0.21 ***	**1**								
Nber_Pan	**0.42 *****	**0.42 *****	**0.60 *****	**1**							
Nber_gr_pan	**0.56 *****	**0.56 *****	0.16	0.14							
Nber_FG_pan	**0.54 *****	**0.55 *****	0.11	0.12							
Gr_Yield	**0.63 *****	**0.63 *****	**0.50 *****	**0.81 *****	**1**						
Diam_Stem	**0.50 *****	**0.50 *****	**0.22 ****	**0.29 *****	**0.48 *****	**1**					
Pl_H	**0.69 *****	**0.69 *****	**0.24 ****	**0.43 *****	**0.66 *****	**0.68 *****	**1**				
Leaf_W	0.10	0.10	-0.02	0.11	0.13	**0.29 ****	**0.20 ***	**1**			
Flag_LW	0.06	0.06	-0.08	0.08	0.08	**0.18 ***	0.15	**0.61 *****	**1**		
Leaf_L	**0.63 *****	**0.62 *****	**0.22 ****	**0.41 *****	**0.59 *****	**0.59 *****	**0.86 *****	**0.26 ****	**0.25 ****	**1**	
Flag_LL	**0.59 *****	**0.59 *****	0.08	**0.32 *****	**0.54 *****	**0.51 *****	**0.76 *****	0.07	0.15	**0.74 *****	**1**
Lig_Leng	**0.49 *****	**0.48 *****	**0.30 *****	**0.25 ****	**0.41 *****	**0.47 *****	**0.58 *****	-0.13	**-0.28 ****	**0.56 *****	**0.45 *****

Axes 1 and 2 of the Principal Component Analysis carried out to describe the grouping of the 136 accessions of rice in Benin with the 14 quantitative variables measured explained together 60.72% of the total available variability (Fig. 2).

All the variables are positively correlated with the first axis except the width of the leaves (Leaf_W) and the width of the panicle leaves (Flag_LL) which are positively correlated with the second axis. The projection of the accessions in the factorial plan formed by the two first axis shows three groups (Fig. 3) that are not however very distinct.

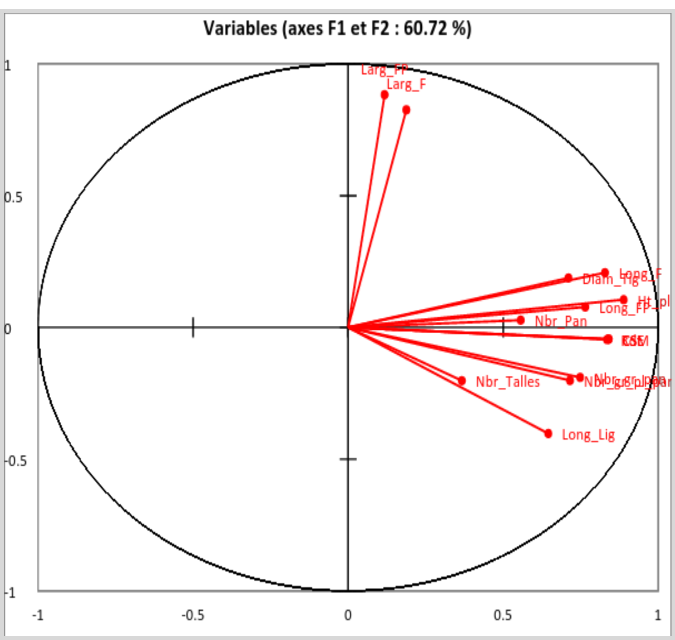

Fig. 2: Correlation circle of the quantitative variables.

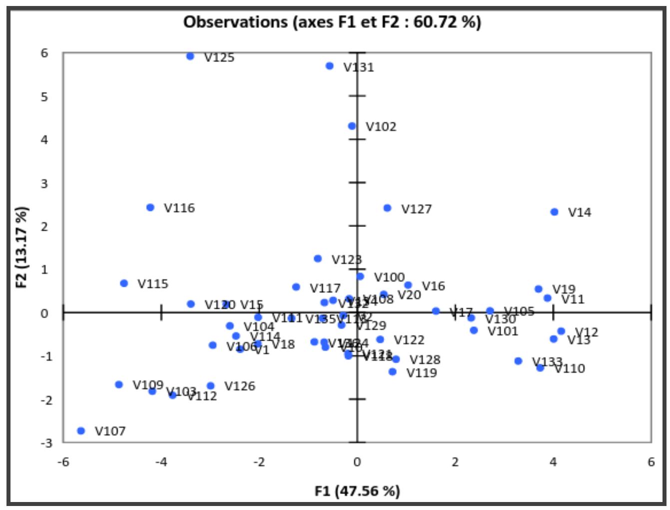

Fig. 3: Grouping of the Benin rice varieties based on Principal Component Analysis.

Cluster analysis based on Euclidean distance classified the 136 varieties into three phenotypic groups (Fig. 4):

- Group 1 (Gp1), containing 27.21% of the varieties, is the one of the early maturing varieties characterized by small diameter, small height. The panicle leaves and leaves of these varieties have narrow width and small length as well as small ligule. They are also characterized by low yields and small numbers of seeds per panicle, full grain per panicle, panicles per plant and tillers per plant. This group contains the checks as T2 (NERICA-L-20), T3 (NERICA-L-19), T5 (WAB 32), T6 (NERICA 4) and T7 (NERICA 2) well as the majority of NERICA hybrids such as NERICA 14, NERICA-L-19 and NERICA 2 collected in the field (Fig. 4).

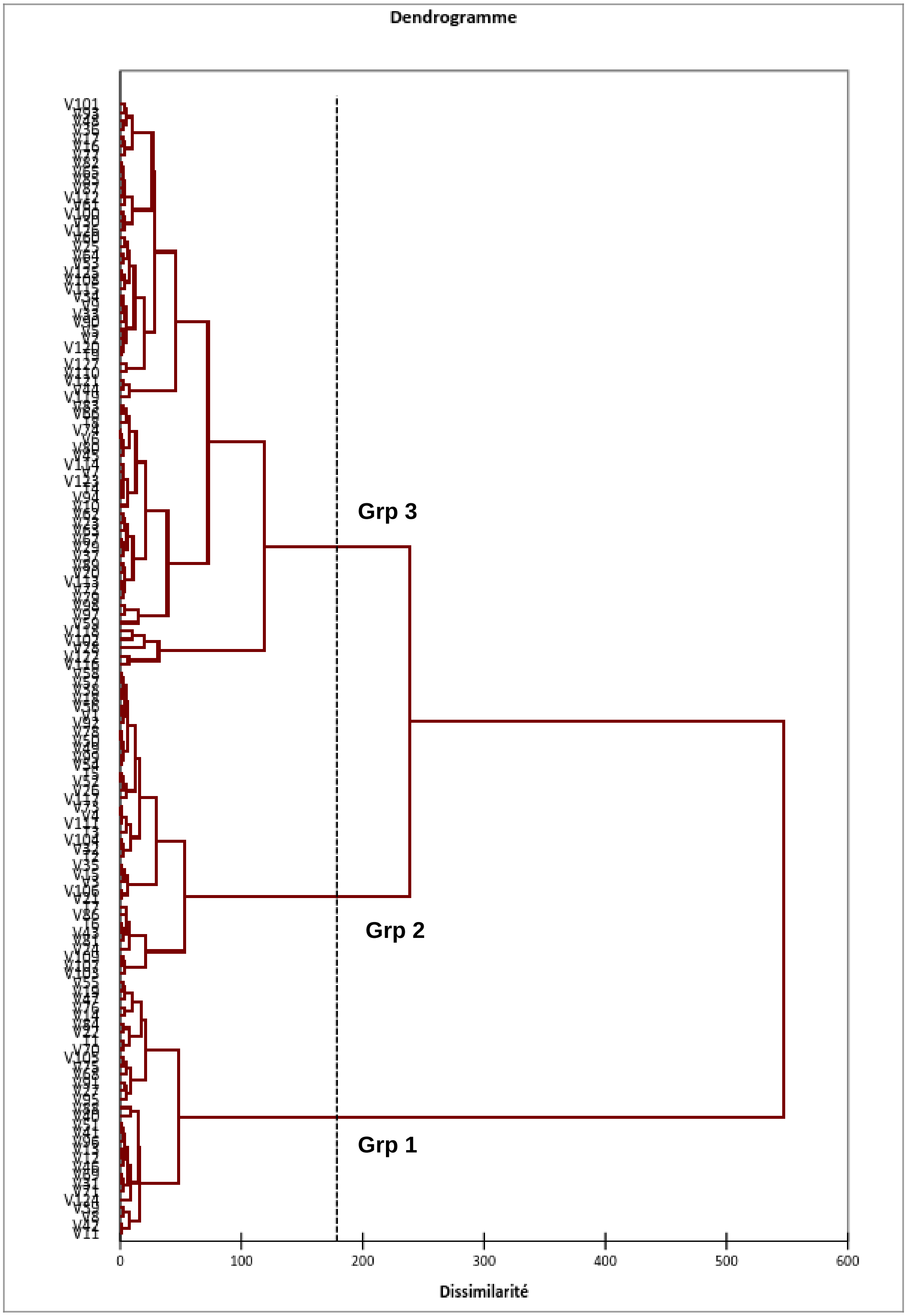

Fig. 4: The different phenotypic groups within the accessions analysed.

- Gp2 group, comprising 50% of the varieties, is intermediate between the other two groups. It is characterized by an intermediate cycle of medium diameter and medium height but the width of leaves and panicle leaves are broad. The length of their leaves, their panicle leaves and their ligule are average. There is also a mean yield in this class characterized by an average number of grains per panicle, full grains per panicle, panicles per plant and tillers. It contains witnesses T4 (ADNI 11), T8 (IR841) and T9 (CG 14) and most *indica* of the collection such as Jasmine, Moépoua and Grand Djimbo (Fig. 4).

- Gp3 consists of late varieties of large diameter and height but of average leaf width and panicle leaf width. They have long length of leaves, panicle leaves and ligule. Their yield is very high as well as the numbers of grains per panicle, full grains per panicle, panicles per plant and tillers. It comprises 22.79% of the varieties. In this class there is the check 1 (Gambiaka kokou) and almost all *japonica* of the collection such as Gambiaka, De Gaulle, Lobolobo, etc. (Fig. 4).

Phenotypic diversity and distribution of rice varieties in Benin

The mean Shannon diversity index (H) calculated for the collection is 0.72 (Table 10) and shows that a high phenotypic diversity exists in the collection but unevenly distributed. The one calculated for the quantitative variables is 0.94 (Table 9) and also shows a high phenotypic but unequally distributed diversity. Indeed, phenotypic diversity is high in zone IV (0.62) but low in other agro-morphological zones. In the phenotypic groups, there is also a high variability in the mean index of diversity. Thus, the phenotypic group Gp2 (0.72) has a high phenotypic diversity whereas diversity is low in the other groups (0.49 for Gp1 and 0.45 for Gp3).

Table 9. Estimates of diversity index of Shannon-Weaver collected on the 14 quantitative variables of the rice collection of Benin, by agro-ecological zones and phenotypic groups detected by the Ascending Hierarchical Classification (AHC).

Quantitative variables	C	Phenotypic groups			Agro-ecological areas							
		Gp1	Gp2	Gp3	I	II	III	IV	V	VI	VII	VIII
Sowing-heading cycle	0.92	0.38	0.59	0.3	0.11	0.32	0.32	0.51	0.34	0.15	0.02	0.04
Sowing-maturity cycle	0.86	0.38	0.56	0.27	0.1	0.3	0.31	0.47	0.35	0.16	0.02	0.04
Stem diameter (cm)	0.95	0.53	0.74	0.43	0.16	0.47	0.39	0.6	0.46	0.23	0.03	0.06
Plant height (cm)	0.86	0.5	0.64	0.31	0.18	0.48	0.36	0.55	0.45	0.24	0.03	0.07
Leaf width (cm)	0.81	0.47	0.75	0.46	0.17	0.46	0.38	0.55	0.43	0.23	0.03	0.06
Flag leaf width (cm)	0.71	0.5	0.63	0.44	0.16	0.44	0.35	0.53	0.42	0.23	0.03	0.06
Leaf length (cm)	0.98	0.44	0.72	0.42	0.17	0.45	0.38	0.61	0.47	0.23	0.03	0.07
Flag leaf length (cm)	0.82	0.4	0.66	0.48	0.16	0.44	0.38	0.55	0.48	0.22	0.03	0.06
Ligule length (cm)	0.93	0.47	0.76	0.48	0.17	0.46	0.38	0.64	0.47	0.2	0.03	0.06
Number of full grain per panicle	0.95	0.49	0.74	0.45	0.17	0.47	0.4	0.62	0.56	0.23	0.03	0.06
Number of grains per panicle	0.95	0.45	0.71	0.45	0.16	0.46	0.4	0.63	0.46	0.23	0.03	0.06
Number of panicles per plant	0.94	0.56	0.75	0.46	0.17	0.47	0.39	0.63	0.44	0.23	0.03	0.07
Number of tillers	0.93	0.53	0.79	0.51	0.17	0.49	0.38	0.63	0.43	0.24	0.03	0.06
Grain yield (g/m^2)	0.94	0.46	0.7	0.44	0.17	0.46	0.4	0.62	0.47	0.23	0.03	0.06
Average (quantitative variables)	0.9	0.47	0.7	0.42	0.16	0.44	0.37	0.58	0.44	0.22	0.03	0.06

Table 10. Estimates of diversity index of Shannon-Weaver collected on the 14 qualitative variables of the rice collection of Benin, by agro-ecological zones and phenotypic groups detected by the Ascending Hierarchical Classification (AHC).

Quantitative variables	C	Phenotypic groups			Agro-ecological areas							
		Gp1	Gp2	Gp3	I	II	III	IV	V	VI	VII	VIII
Color of the base of the plants	0.49	0.39	0.54	0.3	0.1	0.33	0.21	0.5	0.29	0.16	0.03	0.05
Anthocyanin coloration of the leaf sheath	0.49	0.39	0.54	0.27	0.1	0.32	0.21	0.49	0.29	0.16	0.03	0.05
Leaf blade coloring	0.67	0.69	0.86	0.58	0.21	0.6	0.41	0.3	0.57	0.32	0.06	0.09
Distribution of anthocyanin / leaf blade	0.56	0.46	0.62	0.41	0.13	0.39	0.26	0.59	0.37	0.21	0.03	0.06
Color of auricle	0.54	0.39	0.44	0.29	0.12	0.28	0.2	0.46	0.29	0.14	0.02	0.04
Color of necklace	0.54	0.46	0.59	0.41	0.15	0.37	0.26	0.58	0.37	0.2	0.03	0.06
Attitude of leaf blade	0.17	0.38	0.35	0.36	0.13	0.32	0.26	0.39	0.32	0.19	0.03	0.06
Shape of the ligule	0.44	0.44	0.47	0.45	0.15	0.36	0.33	0.46	0.39	0.2	0.03	0.06
Attitude of the panicle leaf	0.51	0.4	0.47	0.35	0.13	0.32	0.28	0.43	0.28	0.18	0.03	0.05
Anthocyanin coloration level of knots	0.39	0.4	0.53	0.34	0.13	0.37	0.26	0.52	0.36	0.17	0.03	0.06
Color Lemma and palea (1)	0.63	0.28	0.48	0.31	0.09	0.27	0.22	0.38	0.29	0.13	0.02	0.03
Color of apex (1)	0.65	0.32	0.49	0.28	0.09	0.29	0.19	0.39	0.26	0.1	0.02	0.03
Attitude of ramifications	0.65	0.36	0.53	0.22	0.11	0.32	0.24	0.37	0.29	0.15	0.02	0.04
Form of the caryopse	0.88	0.38	0.69	0.4	0.14	0.35	0.28	0.5	0.37	0.16	0.02	0.05
Average (qualitative variables)	0.54	0.41	0.54	0.36	0.13	0.35	0.26	0.49	0.34	0.18	0.03	0.05
Average collection (quantitative and qualitative variables)	0.72	0.44	0.62	0.39	0.44	0.39	0.31	0.54	0.39	0.2	0.03	0.06

For the qualitative variables, the accessions of Benin present a high phenotypic diversity (the average index of diversity is 0.54). Despite this high phenotypic diversity within the collection, all agro-ecological zones have a low diversity but variables such as base color of the plants for Zone IV varieties, leaf blade staining of Zones II, IV and V, anthocyanin distribution of the leaf blade of Zone IV, collar color, anthocyanin staining at the nodes of Zone IV, and the shape of the caryopse of Zone IV have a high diversity. However, the Gp2 group (0.54) shows a high phenotypic variability, unlike the other groups (0.41 for Gp1 and 0.36 for Gp3) (Table 10).

Comparisons of the mean of the phenotypic values of the three phenotypic groups (Table 11) show that the three groups are significantly different.

Table 11. Results of comparisons of means of quantitative traits of the three phenotypic groups (Gp) by analysis of variance.

Phenotypic variables	Average phenotypic groups			Risk α of rejection of H_0
	Gp1	Gp2	Gp3	
Sowing-heading cycle	81.59a	92.28b	124.64c	Sowing-heading cycle
Sowing-maturity cycle	113.63a	124.55b	156.16c	Sowing-maturity cycle
Stem diameter (cm)	0.46a	0.5b	0.55c	Stem diameter (cm)
Plant height (cm)	110.84a	141.08b	177.86c	Plant height (cm)
Leaf width (cm)	1.3b	1.46a	1.39ab	Leaf width (cm)
Flag leaf width (cm)	1.49b	1.67a	1.57ab	Flag leaf width (cm)
Leaf length (cm)	37.57c	54.25b	68.7a	Leaf length (cm)
Flag leaf length (cm)	32.58a	43.91b	57.89c	Flag leaf length (cm)
Ligule length (cm)	1.57a	2.07b	2.7c	Ligule length (cm)
Number of full grain per panicle	105.02a	136.76b	161.94c	Number of full grain per panicle
Number of grains per panicle	113.67a	148.71b	175.12c	Number of grains per panicle
Number of panicles per plant	11.77a	13.75b	17.07c	Number of panicles per plant
Number of tillers	17.8b	22.34a	22,48a	Number of tillers
Grain yield (g / m²)	866.77a	1366.48b	2032.63c	Grain yield (g / m²)

Discussion

The phenotypic variability of rice accessions in Benin was evaluated using qualitative and quantitative agro-morphological descriptors according to the recommendations of Sow et al. (2013). Among the 20 quantitative variables 14 are the most discriminating for the different varieties of rice grown in Benin. These are descriptors such as plant height (Pl_H), leaf length (Leaf_L), panicle leaf length (Flag_LL), number of tillers (Nber_Tillers), yield (Gr_Yield), sowing to heading cycle (SHC), sowing-to-maturity cycle (80%SMC), leaf width (Leaf_W), etc. Variables such as leaf length and width have been discriminated by Sié, (1991) through the genetic evaluation of traditional rice varieties in Burkina Faso. Moreover, Moukoumbi et al. (2011) identified the height of the plants, the length and the width of the leaves as discriminating descriptors for lowland NERICAs. For the qualitative descriptors of the 24 variables 14 are the most related and the most discriminating of the different varieties of rice grown in Benin. These included the color of the base of the plants (CBP), the anthocyanin coloration of the leaf sheath (CALS), the leaf blade coloring (LBIC), the distribution of the anthocyanin in the leaf blade (DALBl), etc. These variables according to Moukoumbi et al. (2011) can be used like scorer from the content anthocyanin to select the wanted varieties.

Phenotypic characterization of plants can provide useful information on the structure and the spatial distribution of the diversity. This information contributes to optimum utilization of crop genetic resources. Benin's rice collection is characterized by very late accessions of which 22.79% require more than 145 days from sowing to maturity. The height of the plant varies between 1 and 2.19 m (93.38% of accessions). Sanni et al. (2008) reported that local varieties of Ivory Coast rice were tall and late maturing, while in Burkina Faso most (68%) local varieties of rice showed late maturity (Sie et al., 1998). Sow et al. (2013) also reported the same finding for the Niger collection.

The principal component analysis (PCA) performed with the 14 quantitative variables does not allow the identification of distinct groups. This same observation was made by Sow et al. (2013) in Niger and then by Tendro Radanielina et al. (2013) in the Vakinankaratra region in Madagascar but a clear consolidation pattern was observed for three lines of eight measured on a collection of Spanish barley (Lasa et al., 2001). However, the grouping of the accessions of Benin according to the cluster analysis led to three phenotypic groups. The phenotypic group 1 (Gp1) is that of the improved varieties especially interspecific (NERICA). Thus, in Benin, we see the gradual or even total abandonment of the African species to the detriment of the improved varieties like IR841, NERICA-L-19. This is due to the extent of popularization of these improved varieties in Benin today. Indeed, the variety IR 841 is the most widely grown variety in Benin (Sanni et al., 2011).

Furthermore, according to Sie et al. (1998) the African species is abandoned because of its high rate of spontaneous ginning, low productivity and poor presentation of grain. The phenotypic group 2 (Gp2) contains the varieties of the subspecies *indica*. A similar group was identified by Sow et al. (2013) in Niger but at their level the group contains all varieties of Asian rice. Thus, the phenotypic variability of rice germplasm in Benin is high, irrespective of the phenotypic cluster, collection area or ecology except in the irrigated agrosystem. This could be explained by the existence of the same agrosystems across regions and at the village level. Another explanation could be the good exchange of seeds and the spread of varieties in the rice-growing areas of Benin. Another reason could be the relatively narrow genetic base of the varieties released in the irrigated agrosystem, compared to traditional agrosystems (plain and floating), where old and recent accessions of rice were grown together. The genetic base of irrigated rice in Benin could be expanded using the accessions of lowland and floating compartments in breeding programs. The Gp3 is the group of the most productive varieties but of very seed-maturity cycle. This is the *japonica* group. In the Vakinakaratra region in Madagascar (Tendro et al., 2013), structuring also presented *japonica* group.

The average diversity index of Shannon-Weaver of Benin (0.72) is higher than that calculated for 18 descriptors of 270 rice accessions of Niger (Sow et al., 2013). This index varies when comparing individual descriptors. It is also higher than that calculated using 13 descriptors of 880 rice accessions of Côte d'Ivoire (Sanni et al., 2008). These variations in index between countries could be explained by the species cultivated by the producers, the selected descriptors and the variation within the agrosystems where these species develop. For example, the high value of this index for the pubescence of the leaf blade in the genetic material of Niger is probably due to made that the genetic material of the African Rice can have leaves both bald and hairless (Chang et al., 1977) While Asian rice leaves show a predominance of hair that decreases this index. A large agro-morphological variability is observed in the collection of Rice in Benin regardless of the agro-ecological zone (Sow et al., 2013). Zone IV is the main center for diversification of rice cultivation in Benin. Nevertheless, rice cultivation is developing in all the agro-ecological zones of the country due to the high potential of bottomlands at its disposal (MAEP, 2011). It should be noted, however, that the development of an intensive rice system with the increased popularization of improved varieties has considerably reduced the diversity of African rice varieties in Benin. These varieties could be useful for rice improvement as is the case with the AfricaRice Center where collections are kept for long-term use in breeding programs. It urge for Benin to define a policy of conservation (*in situ* and *ex situ*) of the rice genetic resources to save the little that exists for breeding programs and varietal improvement.

Conclusion

A great morphological diversity has been observed between the rice accessions of Benin, which can be structured into three groups: the least productive group (Gp1), the intermediate group (Gp2) and the most productive group (Gp3). The height of the plants, the number of tillers, the yield and the cycle of maturity are the main characters which make it possible to discriminate the three groups of accessions formed. Also, a great diversity of phenotype is observed through the agro-ecological zones. Zone IV (North East of Benin) is the main area of high diversity in the country. However, the use of molecular markers should make it possible to know global genetic diversity, and to refine the results obtained for a rational use of local germplasm in programs aimed at improving rice varieties in Benin.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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