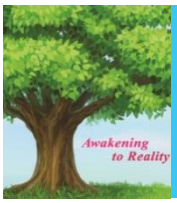

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Morphological Characterization of Five Upland Rice Varieties Grown in Kisangani and its Surroundings, in Democratic Republic of the Congo

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ABSTRACT

The objective of this work was to make an agronomic evaluation and morphological characterization of 4 varieties of upland rice (Lioto, Liboga, Lienge and Nerica7) and 1 traditional variety (Kitombe) in the ecological conditions of Kisangani. To achieve this objective, two trials were conducted at Kisangani in a randomized complete block device (RBCD) with 4 repetitions and 5 treatments (varieties). The Qualitative traits were evaluated by using the Standard Evaluation System for Rice (SES). Quantitative trait analysis showed the statistical difference between varieties ($p<0.05$), ranging from 145±10 to 119±13 for total spikelets/panicle, from 215±6.5 to 318±31.5 for panicle/m^2 and 112±5 to 143±6 for spikelets filled/panicle. Among the qualitative points common to all 5 varieties we found the ligule shape, shape and panicle habit. All varieties have a pointed ligule with two slits, panicle with secondary branches and drooping port. We had identified the proportion of teeth on the leaf. Leaf blade as a new distinguishing feature of rice varieties. The variety Kitombe and Nerica7 have a denticulation on 2/3 of the Leaf blade length against 1/3 for other varieties.

Introduction

Information on genetic diversity and the relationship between different varieties is essential to plant breeders for efficient plant breeding. The estimated genetic similarity between different cultivars can provide help for selecting parents to maintain genetic diversity in an breeding program (Rabara *et al*.,2014). Thus, since the gene is the basic unit of selection and evolution, the measurement of diversity at this scale using molecular markers provides the most accurate information on genetic diversity. The indice of genetic diversity developed in the context of studies of genetic of population (total diversity, subpopulation differentiation, intra-population diversity, heterosis, etc.) are the most relevant indicators. The most indicator widely used for genetic diversity in situ remains the number of varieties. Several studies have shown that local varieties of autogam or vegetatively propagated species have intra-varietal variability and that this is shaped by the species' biology and variety and seed management practices (Maiko *et al*.,2009)

The analysis of phenotypic diversity, for traits related to the polymorphism of major genes (colors, shape and pubescence of certain organs, etc.), cultural constraints (phenology, resistance to biotic and abiotic stresses, response to mineral fertilization, etc.) and use values (culinary quality, nutritive, etc.) can provide relevant indicators of intra-varietal genetic diversity (Radanielina, 2010). It is in this context of phenotypic diversity analysis that this study is carried out in order to provide the relevant information for to improve rice by dreeding in the Democratic Republic of Congo.

Material Et Method

Biological material

Five upland rice varieties were used for this reseach. Four are improved varieties : Lioto (short duration), Liboga (short duration), Lienge (Medium duration), Nerica7, imported (medium duration) and one, Kitombe was traditional variety (Long duration). The seeds were got to National Instute for study and agronomic research (INERA), Yangambi station.

Experimental design

Two trials were conducted on the medical plateau, about 3 km from downtown Kisangani (25°11'East and 0°31'North, 410 m), in the Democratic Republic of the Congo. The climate of Kisangani is Af type according to Köppen classification. It is hot and humid with an average temperature around 24-25°C and a relative humidity of 80 to 90%. The rainfall are distributed almost all the year but the peak is in October and can reach annually a height of 1800 mm and 1925 hours of sunshine. The randomized complete blocks device (RCBD) with 4 repetitions and 5 treatments (varieties) was adopted. Each block (repetition) had 5 elementaries plots (E.P) of 4mx3m, corresponding to the varieties tested. The E.P were separated by 0.5 m while the blocks were separated by 1m. Seed preparation consisted to make the laboratory germination test in Petri dishes and a densimetric sorting which consisted of

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dipping the seeds in tap water for 5 minutes, the floating seeds were discarded. The seedling was direct, manually carried out on 25cmx20cm spacings with 4 grains per poquet. No one mineral fertilization was performed in these trials as the analysis of photosynthetic leaf activity by the Leaf chart color (LCC, Ltd, Japan) method showed no need for fertilization. The experiment soil was analyzed and its approximate composition is presented in Table 1

Data collect and their analyze

Two international descriptors were used for morphological caracteristics. The standard evaluation stystem for rice (SES) provide by International Rice Research Institute (IRRI,2002) and the manual of technician (WARDA, 2009). The photosynthetic activity was estimated by analysis the green color of leaf by using Leaf Color Chart, commonly used in Japan and Philippines, which has 7 coding levels proportional to intensity of the green color of leaves : 1=very clear green, 2=clear green, 3= moderately clear green, 4 = medium green, 5=dark green; 6-7 = very dark green. With 3.5 as the critical value below witch the nitrogen fertilization is required. Other agronomic parameters were observed :

Percentage of germination

It was evaluted at 5 et 7 days afetr seedling (DAS) by the relation :

$$\textbf{Germination}\ (\%) = \frac{\textbf{Number of plants germineted}}{\textbf{Total plants sown}}\textbf{x100}$$

Vegetative vigor (Vg) and emergence vigor (Vm)

Vegetative vigor indicates the number of small leaves produced at 20 DAS (day after seedling) while emergence vigor indicates the size reached by rice plants at 2-leaf stage (approximately 10 DAS). These observations were made on an average of 10 plants in each E.P (n=40)

Tillers (TAL)

It refers to the number of secondary stems (tillers) produced in a square meter chosen at the center of the plot. It was observed at maturity on a sample of $1m^2$/ E.P (n = 4). Tiller fertility (FERT) of each variety was evaluated after harvest in $1m^2$ by the ratio of the number of panicle harvested (PANI) and the total number of tillers (TAL) in that square. The relation used is summarized by the formula of Kouakou et al. (2016) as follows :

$$\textbf{FERT}(\%) = \frac{\textbf{PANI}}{\textbf{TAL}}\textbf{x100}$$

Panicle fertility

After panicle harvest, we count total number of spikelets (grains) per panicle (total EPIL), number of empty spikelets per panicle (EPIV) and number of spikelets filled to determine the percentage of panicle fertility (FERP) and percentage of panicle sterility (STERIL): Fertility and panicle infertility were calculated by the relationship of Kouakou et al (2016) as follows

$$\textbf{FERP}(\%) = \frac{\textbf{EPIL}}{\textbf{Total EPIL}}\textbf{x100}$$

$$\textbf{STERIL}(\%) = \frac{\textbf{EPIV}}{\textbf{Total EPIL}}\textbf{x100}$$

The data obtained were subjected to 5% analysis of variance for the comparison of averages using manual calculation. When differences are observed, multiple comparisons of averages were made to derive homogeneous and heterogeneous groups by calculating the smallest significant difference**.**

Results and Discussion

Morphological characteristics

This study of morphological characterization and agronomic evaluation carried out in the field made it possible to understand better the agronomic behavior and the morphological characteristics of the traditional variety (Kitombe), which was hitherto undescribed, and the four improved varieties (Lioto, Liboga,Lienge et nerica7) in the ecological conditions of Kisangani, because, according to Londo and Schal (2007) and Maiko et al. (2009) some rice characters (e.g grain colors) may change when the same varieties are grown in different areas. Observations made, it emerges that the shape of certain organs (vegetative and generative) of varieties studied have some points of resemblance, This is the case of characters panicle exertion, presence of secondary branches, panicle type, panicle branching port relative to the stem and ligule shape. All varieties have a pointed two-slit ligule, panicle type 1, with secondary branch and good panicle exertion (panicle protruding into the sheath). On the other hand, if one refers to the guiding principles of UPOV, used for the conduct of the examination of distinction, homogeneity and stability (DHS), one notes that the differences exist for the characters such as stigma color and wearing the last leaf (panicle leaf or flag leaf)

These traits are reassuring to distinguish varieties and can therefore be used for the genetic study of traits transmitted to offspring after crossbreeding (Radanielina, 2010). For rice panicle leaf position is great importance. The upright leaves allow the rice to perform intense photosynthetic activity compared to the falling leaf varieties. The mixing of genes by the natural flow of pollen can contribute to the approximation of certain characters observed between varieties because, when several varieties, cultivated and even spontaneous forms coexist, there is crossbreeding and coevolution supported by gene flows and cultural practices. For rice, autogamy is the rule, fertilization takes place before anthesis, this phenomenon is called "cleistogamy"

Nevertheless, there is always a low percentage of allogamy (Gaouna *et al*.,2012) that occurs to a certain extent depending to climatic conditions, cultural practices, the varieties involved and the seedling proximity between two varieties (Gealy *et al*.,2003). The crossing rate due to cross-pollination can range from 0.011 to 0.046% between the rice grown and the adventitious rice (Chen *et al*.,2002), or up to 2.94% between the cultivated varieties (Gaouna et al.,2012). In an study conducted (unpublished) in territory Opala, Liesse at al. (2018) found that the traditional variety Kitombe was the most cultivated as the improved varieties. Among the reasons of choice of this variety by the famers, these author highlights the taste, its swelling and its resistance to stock insects. The presence of arists (beards) and hair on the grains would confer to this variety Kitombe respectively tolerance to birds and insects.

Apart the characters of description of rice varieties know in the inetenational descriptor, we identified in this study that the shape of the leaf blade edge is also an important distinguishing character to consider in the description of rice varieties (Table 3). There are non denticulate varieties at the edge of their leaf blade and denticulate varieties at the edge of their leaf blade . The distribution of teeth on the leaf blade is also a function of the varieties. Some varieties are denticulate at 1/3 of leaf blade (Liboga, Lienge and Lioto) and those

denticulated at 2/3 (Nerica7 and Kitombe). This characteristic is to be observed from stage 6 to 8

Percentage of germination

The emergence of rice starts from 5 days afetr seedling according to the climatic conditions (the humidity and the temperature in particular). Seed dormancy is a very important factor affecting the emergence of seeds from several crops, particularly rice (Dedi *et al*.,2017).

The germination results show that all seeds were good quality because their emergence varies from 80 to 95% according to the varieties (figure 1). In the field, however, a few cases of mortality was observed, which reduced the rate to 7JAS from 80% to 74% for Liboga variety. Currently, the official germination capacity standards (GC) for seed are based on normal plants germineted in the order of 85% for cereals in general (Dedi *et al*.,2017).

It is recognized that a good quality seed must be harvested at physiological maturity (when approximately 85% of grains in panicle turn from green to yellowish) and must not be beaten to prevent grains shock, and must be kept with a humidity not exceeding 13% (Dedi *et al*.,2017). In the absence of adequate storage facilities for long periods of time (cryoconservation at -196°C in liquid nitrogen), it is recommended to sow seeds in the conservative collection every year with regular purifications because the germinability of the rice is deteriorates badly from 8 months after harvest especially when the conditions of conservation are not respected (Dedi *et al.*,2015).

Number of spikelets per panicle (Total EPIL)

The results show that the number of grains in a panicle varies with variety ($p<0.05$). It varies from 112±5.00 (Kitombe) to 143±6.00 (Lienge). In rice, the number of grains per panicle is generally ranges from 25 to 360 with an average of 100 to 150 (Kasongo *et al*.,2003) and is not necessarily positively correlated with panicle length.

Since the number of grains per panicle is one of the major components of rice yield (Ondo *et al.*,2013), it is likely that Lienge and Kitombe varieties are the most productive and the least productive respectively if other yield parameters such as number of panicle per square meter, weight of thousand grains, etc.) evolve in the same direction. The number of grains per panicle is a varietal character (Liboga *et al*.,2018), but it should be noted that environmental factors such as soil fertility, climatic conditions (rainfall, temperature) can play a major role in the number of grains produced in a panicle.

Fertility of panicles (FERP %)

The panicle fertility is the percentage ratio between the number of grains (spikelets) filled and the total number of grains. While the percentage of sterility or infertility is the ratio of the number of empty grains to the total number of grains (Kouakou *et al.*,2016). The damage caused by the birds is not in this case to be considered. In this study, panicle fertility was found vary eccoding to variety ($p <0.05$) and ranged from 91% (Liboga) to 98% (Lienge).

In accordance to standard evaluation system for rice (SES) of international rice research institute (IRRI,2012), Lienge, Nerica7 and Lioto varieties with 95% fertility are considered fertile,while Kitombe and Liboga, whose fertility is below not 95% are considered moderately fertile. Courtois and Taillebois (1990) found in a study of the endogenous behavior of some lines that male sterility of cytoplasmic origin also causes the abortion of microspores. However, abiotic factors such as absence of rainfall and the presence of strong winds causing lodging of plants before the filling phase and biotic factors such as biting-sucking insects, ants that devour the anthers at flowering can significantly affect the fertility of rice (Liboga *et al.*,2018) and can reach 18% in certain situations (Amir *et al*.,2018)

Panicle length (Pl)

There is statistic difference between varities ($p<0.05$). The length varies from 25.5cm (Nerica7) to 31.08cm (Kitombe) as shown in Table 2. In general, a rice panicle measures 15 to 50cm with a average oscillating between 20 to 24cm (Kasongo e*t al*.,2003). By a visual comparison with the number of grains per panicle, it has been found that a long panicle does not necessarily have a high grain number, which shows that our results correspond with those obtained by Kasongo *et al*.(2003) in a comparison study of hybrid lines at Yangambi.

However, Ondo *et al.* (2013) indicate that rice varieties with abundant tillering, vigorous stems, high numbers of spikelets and long panicles are generally considered productive varieties.

Vegetative vigor (Vg) and emergence vigor (Vem)

These two types of vigor were evaluated according to the WARDA rating scale (WARDA, 2009). All varieties were rated 5, which means that they have good growth at 10-20 DAS stage. Although vegetative development is not always directly correlated with yield in rice (Ondo *et al*.,2013), we do believe that it is highly likely that varieties with good vegetative growth will produce better because vigorous growth stifles competing weeds (Liboga *et al.*, 2018). The weed is one of the major biotic constraints of upland rice cultivation in the tropics (Jamil *et al*.,2012), especially in a grassy fallow.

Panicle number and tillers fertility (% FERT)

It follows from our study that the number of panicles produced per square meter is significantly a function of variety. It ranges from 215.5 (Kitombe) to 318.5 (Lienge) as shown in Table 2

The difference in the number of panicles per square meter observed between varieties can be attributed to the genetic potential of each variety. Indeed, the variety Lienge is an improved variety, created at Yangambi station (Mateso *et al*.,2003a) to have of high yield, it is not surprising that this variety is more productive in number of panicle per meter square compared to Liboga and Lioto varieties that were developed in the same station to have a short duration (90 days) (Mateso *et al.*,2003b). On the other hand, the behavior of Nerica7 in terms of panicle is justified by the fact that this variety has benefited from interesting additive effects coming from the interspecific crossing between African rice (*Oryza glabberima* Steud.) And Asian rice (*Oryza sativa* L.). There is no significant difference between varieties tested for tiller fertility. The fertility found varies from 98 to 100%. In general, a rice plant can produce about hundred tillers that can be fertile or not, however, excessive tillering often makes for poorly uniform maturation and is recommended for well-fertilized, sunny, irrigated and mechanized crops. Only around 20-40% of these tillers develop and are exploited by human, especially in the Democratic Republic of Congo, where upland rice is predominantly cutiveted. Other tillers remain dormant, especially in Asian rice, so it is often found to regrow a few days after harvest and it is in this case that asian rice (*O. sativa L.*) is considered as perennial plant thus differentiating it from African rice *(O.glabberima*) which is totally annual.

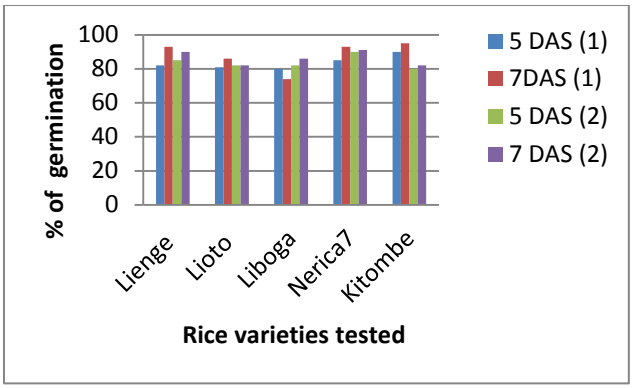

Figure 1. Emergence evolution at 5 et 7 DAS in the first and the second experiment.

Table 1. Approximate composition of the experiment soil

	Clay			pH		
Soil depth (cm)	0-10	10-20	20- 30	0 – 10	10-20	20- 30
Content (%)	19,2	20,8	27,2	5,26	5,36	5,39
	silt				Total nitrogen	
Soil depth (cm)	0-10	10-20	20-30	0-10	10-20	20- 30
Content (%)	8	8.8	5.6	0,12	0,036	0,046
	Sand			Total organic carbon		
Soil depth (cm)	0-10	10-20	20- 30	0-10	10-20	20-30
Content (%)	72,8	70,4	75,2	1,88	1,7	1,82

Table 2. Panicle characteristics

Varieties	Ram.	Pl (cm)	Total EPIL	EPIL	EPIV	FERP (%)
Kitombe	12±1b	31,08±1,50b	119±13d	112±5,00d	7± 2b	94,11±7,02b
Lienge	15±2a	29,17±2,30a	145±10a	143±6,00a	2±2d	98,62±4,3a
Nerica7	12±1b	25,50±1,60c	139±11b	134±5,00b	5±3c	96,40±6,12ab
Lioto	12±1b	26,91±1,20c	136±13b	134±3,00b	2±1d	98,52±3,10a
Liboga	11±1b	26,9±1,50c	130±11c	119± 3,00c	11± 3a	91,53± 2,02b
Average	10,00	27,57	133,80	128,400	3,40	96,52
DL (variety)	4	4	4	4	4	4
CV (%)	8,4	13,2	14,13	22,3	8,9	16,40
LSD 0,05	2,9	1,95	4,70	6,91	1,96	3,30

* On the same column, values followed by the same letters are not statistically different (α=5%)

Table 3. Tillers characteristics and plant vigor

Varieties	PANI.m^{-2}	FERT%	Vg. (1-9)[a]	Vem. (3-7)[a]
Kitombe	215,5± 6,50e	98±1a	5	5
Lienge	318,5±31,50a	100a	5	5
Nerica7	307,75±16,25b	100a	5	5
Lioto	273,75±14,37c	99,0±1a	5	5
Liboga	269,5±26,50c	99,5±1a	5	5
Average	307,4	99.3	5	5
DL (variety)	4	4	4	4
LSD 0,05	10,5	2,94	-	-

a=Visual observation based on Manual of technician, (WARDA, 2009). On the same Column, values followed by the same letters are not statistically different (α=5% ***)***

Table 4. Morphological characters

	Varieties tested					
Observed parameters	**Lienge**	**Lioto**	**Liboga**	**Nerica7**	**Kitombe**	**S.O**
Anthocyanin pigmentation de coleoptile (1)	very weak	very weak	very weak	very weak	weak	0-1
Leaf gain Color (basal Leaf (2)	Green	Green	green	green	Violet-claire	5-7
Intensity of green leaf color (3a)	Medium	Dark	Dark	Medium	Medium	5-7
Anthocyanin Pigmentation of Leaf (4)	Present	Present	Present	Present	Present	5-6
Distribution of pigmentation leaf anthocyanin (5)	Uniform	Uniform	Uniform	Uniform	Uniform	5-6
Anthocyanin pigmentation of gain (6)	Absent	Absent	Absent	Absent	Present	7-8
Intensity of anthocyanin coloration (7)	-		-	-	weak	5-6
Leaf Pubescence (8)	Nothing	weak	weak	weak	High	6-9
Pigmentation anthocyanin auricles (9)	Absent	Absent	Absent	Absent	Present	7-8
Edge of limb (10)	denticulate	denticulate	denticulate	denticulate	denticulate	6-8
Distribution of teeth on the limb edge [1]	1/3 denticulate	1/3 denticulate	1/3denticulate	2/3 denticulate	2/3 denticulate	6-9
Ligule shap (11)	pointed	pointed	pointed	pointed	Pointed	6-7

Ligule color (12)	Colorless	Colorless	Colorless	Colorless	clear purple	6-7
Leaf length (limb) (13)	Medium	Medium	Medium	Medium	Medium	7-8
Leaf width (limb) (14)	Medium	Medium	Medium	Medium	Medium	7-8
* pigmentation anthocyanin de stigma (15)	very weak	very weak	very weak	very weak	Medium	6
*Port of flag leaf (panicle leaf (16)	Half-erect	Erect	Erect	half-erect	Curved	7-9
Port of stem (17)	Half-erect	Erect	Erect	Erect	Half-erect	7-9
Pigmentation anthocyanin of Lent (lemma less) (18)	Nothing	Weak	Weak	Nothing	High	9
heading epoch (19)	Medium	early	early	early	Late	9
Anthocyanin coloration of the area under the apex (lemma) (20)	Very weak	Very weak	weak	Very weak	weak	9
* Pigmentation anthocyanin of apex (lower lemma) (21)	Very weak	Absent	Very weak	Very weak	Medium	9
*stigma color (22)	green clear	green clear	green clear	green clear	purple	7
* Anthocyanin coloration of the nodes (23)	Absent	Absent	Absent	Absent	Present	6-8
Intensity of pigmentation anthocyanin of the nodes (24)	Weak	Weak	Weak	Weak	Medium	6-8
Intensity of pigmentation anthocyanin internode (25)	Very weak	Very weak	Very weak	Very weak	Very weak	6-8
Color of tuft collar at tillering (26)	green clear	green clear	green clear	green clear	Purple	3
Intensity of anthocyanin color from neck to tillering (27)	Very weak	Very weak	Very weak	Very weak	High	3
Edges (27)	Absent	Absent	Absent	Absent	Present	9
Edges color (late observation) (28)	-	-	-	-	Reddish-brown	9
* Edges distribution (29)	-	-	-	-	On1/4> only	8-9
Length of the longest edges (30)	-	-	-	-	Medium	9
*lemmot Pubescence (31)	Absent	present	present	Absent	Present	8-9
Panicle exertion (32)	Well Salient	Well Salient	Well Salient	Well Salient	Well Salient	7-9
Color of the lemmotop (33)	purple	purple	purple	purple	purple	9
* Port relative to the stem (34)	Half-erect	Erect	Erect	Half-erect	Slightly drooping	8-9
Presence of secondary branches (35)	Present	Present	Present	Present	Present	8-9
Secondary branching type (36)	Type 1	Type 1	Type 1	Type 1	Type 1	8-9
Panicle port in relation to the stem (37)	falling	falling	falling	falling	falling	7-9
Phenotypic acceptability (38)	excellent	excellent	excellent	excellent	Good	7-9
Epoch of leaf senescence (39)	intermediate	late	Late	intermediate	precocious	9
Glume length (34)	Medium	Medium	Medium	Medium	Short	9
glume color (41)	Purple	Purple	Red	Purple	straw color	9
Grain width (42)	Medium	Medium	Medium	Medium	Medium	9
* Length of caryopsis (43)	Medium	Medium	Medium	Medium	Short	9
Width of caryopsis (44)	Medium	Medium	Medium	Medium	Medium	9
Capucule position (45)	Horizontal	Oblique	Oblique	Horizontal	oblique	9
Caryopsis shap (46)	half fusiform	Fusiform	Fusiform	Half fusiform	Half rounded	9
Caryopsis color (47)	White	White	White	White	red clear	9
* Caryopsis Aroma (48)	Absent	Absent	Absent	Absent	Weak	9
Panicle egrenability (49)	Easy	Difficult	Difficult	Easy	Difficult	9
Lodging resistence (50)	Medium	Medium	Medium	Medium	Medium	6-9
Digestion of alkalis (51)	Intermediate	IIntermediate	High	low	Low	9

*=UPOV guiding principles for Conduct of the Examination of Distinctness, Uniformity and Stability of Rice varieties; 1-50=Visual observation based on manual of technician (WARDA, 2009) and/or standard evaluation system for rice (SES) (IRRI, 2002); a: Observation based on the Leaf Color Chart., ([1]): New criterion in the description of rice varieties; S.O = stage of observation

Conclusion

Characterization of genetic material is important for the conservation of genetic resources for effective conservation management and efficient use especially in breeding programs.

We are interested in carrying out this study as part of our rice research program where we previously determined the physicochemical and technological characteristics of these same varieties. In this chapter, these rice varieties were evaluated in the field and morphologically characterized to assess their phenotypic diversity.

Examination of this morphological diversity showed that the qualitative traits exhibited moderate diversity. In fact we know that rice is autogamy but there is always a small percentage of allogamy influenced by the environment, by varieties and technical pratices.

As a result, gene exchange is possible through pollen flow and often creates an undesirable crossing. It is therefore important to regularly purge the field to maintain varietal purity.

Among the qualitative points common to all varieties, the ligule shap and panicle shap were noted. All varieties have a pointed ligule with two slits, panicle with secondary branches. The stigma of the Kitombe variety is purple and its spikelets are aristate while the stigmas of the improved varieties are colorless and the spikelets are without barbs. This characterization study made it possible to describe the varieties studied and this description can be valorized if we wish to make crossings between these varieties. In this

description, we had identified a new criterion of characterization of the rice which is the proportion of teeth on Leaf blade. It should be noted that the variability within cultivated rice varieties is sometimes more complex than it is necessary to carry out further studies, integrating molecular tools experiments such as microsatellites or RFLP markers.

According to the standard evaluation system for rice (SES) the varieties Lienge, Nerica7 and Lioto whose fertility reaches 95% are considered fertile while Kitombe and Liboga whose fertility does not reach 95% are considered moderately fertile. This allows to conclude partially that despite the differences noted, all our materials have a good level of panicle fertility

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