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Genetic diversity studies on selected rice varieties grown in Africa based on aroma, cooking and eating quality

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Rice grain quality is an important factor that has a great influence on its market value and consumer acceptance. It is determined by three parameters controlling the cooking and eating qualities of rice (amylose content, gelatinization temperature and gel consistency) and by the aroma, which becomes a criterion increasingly preferred by consumers. Molecular characterization of specific genomic regions of rice genotypes by trait specific markers can help in the development of suitable breeding program. This study was conducted at AfricaRice Regional station, Saint-Louis, Senegal. 30 rice genotypes commonly used in Africa were evaluated using eight simple sequence repeat (SSR) markers linked to the cooking, eating properties, and the aroma. The total number of alleles was 45 with an average of 5.63 allele per locus. The number of alleles per marker varied from three for RM204 to eight for RM190 and RM342A and the effective number of alleles varied from 1.66 for RM204 to 6.16 for RM342A. The polymorphic information content (PIC) varied from 0.39 to 0.83 and the allele frequency ranged from 0.015 to 0.75. A maximum genetic similarity of 1 was observed between Gambiaka Kokoum and Gambiaka Burkina Faso, Basmati 270 and Basmati 370, Sahel 108 and Sahel 201, Sahel 108 and Sahel 208, Sahel 201 and Sahel 208, Sahel 202 and Sahel 209, and Sahel 305 and Sahel 317. The Sahel varieties found with maximum genetic similarity have the same amylose content, but different gelatinization temperature except Sahel 305 and Sahel 317 which have the same cooking and eating properties. Therefore, more markers are needed to discriminate those varieties. Minimum genetic similarity was observed between traditional aromatic rice Basmati 370 and the landrace Gambiaka Nigeria. The unweighted pair-groups method using arithmetic averages (UPGMA) cluster analysis of these cultivars enabled the classification of our varieties in five major groups with additional sub-clusters in groups 2, 3 and 4. Groups 1 and 2 composed of aromatics varieties, group 3 gathered the three improved Sahel aromatic varieties, group 4 was the most diversified group with three sub-clusters and group 5 corresponded to the traditional varieties Gambiaka. The results of this study indicated that the use of trait specific SSR markers enabled to group the varieties according to their cooking and eating quality and the aroma and therefore can be very useful in breeding rice varieties harboring good cooking and eating quality traits and aroma in rice breeding program.

Key words: Grain quality, cooking and eating properties, aroma, cluster analysis, simple sequence repeat (SSR), rice.

INTRODUCTION

The genus *Oryza* of the Graminae family has 22 species among which only two *Oryza glaberrima* domesticated in Africa and *Oryza sativa* domesticated in South Asia are cultivated around the world (Bounphanousay et al., 2008). *O. sativa* L. is considered as one of the major cereal crops with agronomic and nutritional importance. It is a staple food for more than a half of the world's population and accounts for 21% of global human per capita energy, 15% of per capita protein (Maclean et al., 2002) and 21% fat supply (Kennedy and Burlingame, 2003).

The grain quality of rice is an important factor that has a great influence on its market value and consumer acceptance (Demont et al., 2012); it includes a range of parameters such as appearance, milling quality and physico-chemical properties. The latter ones are reported to be very important for consumer and market place (Juliano and Perez, 1988). It is determined by three physicochemical properties, that is, the amylose content (AC), the gelatinization temperature (GT) and the gel consistency (GC). Aromatic rice are very popular in Africa, because of their flavor and texture, which makes aroma a criterion increasingly preferred by consumers.

Rice grain quality is difficult to define, because of quality varying preferences depending on the cooking culture. For example, in Japonica rice eating countries, rice with short grains and low amylose content are preferred, because they become soft and sticky after cooking (Danbaba et al., 2011). However, in *indica* consuming countries including most of the African countries and Pakistan, long grain rice with intermediate AC and GT are preferred because they become soft and fluffy after cooking. Rice grain is mainly composed of starch which consists of two forms of glucose polymers, namely, amylose and amylopectin and therefore its cooking and eating characteristics are mainly assessed using its AC, GT and GC (McKenzie and Rutger, 1983). AC is considered as the most important criteria influencing the behavior of rice during cooking and processing (Juliano, 1979; Webb et al., 1985). It is correlated with the increase in volume, water absorption during cooking and the hardness and whiteness of cooked rice (Juliano, 1985). Based on AC, rice is classified as: Waxy rice (0 to 2% AC) and non-waxy rice which are divided into very low AC (3 to 9%), rice with low AC (10 to 20%), intermediary AC (20 to 25%) and high AC (above 25%) (Bao et al., 2006; Wani et al., 2012). Rice with high AC becomes hard after cooking (Rao et al., 1952; Williams et al., 1958). A gene named waxy gene on the short arm of chromosome 6 explained most of the variations observed in AC among cultivars and many markers present on this short arm have been mapped to quantitative trait loci (QTLs) with major effects controlling AC (Septiningsih et al., 2003; Aluko et al., 2004; Fan et al., 2005; Shu et al., 2006). The GT reflects the ease or difficulty to cook the rice (Bao et al., 2007); it is related to the chain length distribution of amylopectin (Bao et al., 2009; Noda et al., 2003). It is defined by the alkali spreading value (ASV) based on the degree of diffusion of six grains of rice in a solution of KOH at 1.7%. Rice grains with a high GT remain unchanged, while those with low GT are completely disintegrated and the ones with medium GT are partially affected. Alkali spreading value corresponds to GT as follows: ASV of 1 to 2 for high GT (74.5 to 80°C), 3 for high intermediate GT, 4 to 5 for intermediate GT (70 to 74°C), and 6 to 7 for low GT (<70°C). GT is controlled by the *ALK* gene responsible for the synthesis of the enzyme soluble starch synthase sub-type IIa (SSIIa) (Umemoto et al., 2002). A major QTL controlling GT was located in the alk locus region near the waxy gene (3.93 cM) (Tan et al., 1999). This locus was also detected by Shu et al. (2006) in addition to a second QTL detected in the *alk* locus region linked with the marker RM276. The GC is a measure of firmness of the rice after cooking and is performed to classify rice with the same amylose content, particularly those with high AC into hard, medium or soft texture (Sabouri et al., 2012). Two QTLs controlling GC were detected on chromosome 6 (Lanceras et al., 2000; Tian et al., 2005). GC QTLs have been reported to be linked to *wx* locus (Fan et al., 2005; Fitzgerald et al., 2008; Tan et al., 1999). Some research works on loci controlling rice physico-chemical properties have reported that the three parameters are under the control of the waxy locus or a genomic region closely related to this locus (Tan et al., 1999)

Aroma also called fragrance has become an important criterion in the selection of rice due to high consumer preference for aromatic rice. It plays an important role in rice price. In Africa, aromatic rice from Pakistan (Basmati) has a high market price. High milling returns and good cooking quality are often associated with aromatic rice (Tripathi and Rao, 1979; Sattari et al., 2015). Fragrance is mainly due to the presence of a compound named 2-acetyl-1-pyrroline (2AP) (Lorieux et al., 1996). 2Ap is produced by a deletion of 8 bp of exon 7 on rice chromosome 8 leading to a recessive allele *fgr* coding for a nonfunctional betaine aldehyde dehydrogenase (badh2) (Bradbury et al., 2005). Many inexpensive, simple and rapid polymerase chain reaction (PCR)-based markers, such as SNP, SSRs markers linked to aroma have been developed (Cordeiro et al., 2002). RM 515,

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RM 223 and RM 342A are located on chromosome 8 and explained 41.78, 28.38, and 22.46% of the phenotypic variation, respectively (Kibria et al., 2008).

Afirca Rice Center has created and released number of varieties in Senegal, Mauritania, Mali and Gambia. The released varieties included interspecific and aromatic with variable cooking and eating properties. However, breeding programs aiming to develop rice varieties with good cooking, eating qualities and aroma are difficult, due to the polygenic inheritance and the environmental interactions (Lapitan et al., 2007; Lestari et al., 2009). Therefore, precise information on genetic diversity of the rice genome regions controlling these parameters will be useful for efficient breeding programs. These information can be provided by SSR markers which have been used successfully for genetic diversity studies due to their multiallelic nature, high reproductibility, co-dominant inheritance, abundance and extensive genome coverage (Sivaranjani et al., 2010). In this study 30 varieties including Africa rice released varieties and some others commonly grown rice varieties in Africa were evaluated for their genetic diversity using SSR markers that are genetically linked to aroma and QTLs controlling rice cooking and eating properties.

MATERIALS AND METHODS

Plant

The research was undertaken in 2012 at the AfricaRice Regional station of Sahel (Saint-louis-Senegal). Thirty varieties (Table 1) were investigated, including 4 interspecific NERICA varieties created by AfricaRice Sahel station, 15 improved Sahel varieties of different origins mostly released and cultivated in Senegal, Mauritania and Gambia, 5 traditional aromatic varieties including, 3 Basmati genotypes, Dom-Seophid, and KDM105 having a high market value, 4 landraces Gambiaka well spread in Africa, TS2 a Taiwanese variety released and cultivated in Burkina Faso and Wab 638-1 bred in Ivory Coast at ADRAO center (former name of AfricaRice); the seeds for all the varieties were provided by AfricaRice center. Ten seeds of each variety were pre-germinated in Petri dishes and then transferred in pots containing clay as substrate and placed in the screen house 3 weeks later. Leaves were collected from 45 days old seedlings for DNA extraction.

DNA extraction

DNA was extracted from the leaves of 45-day-old seedlings using the Dellaporta protocol with some modifications (Dellaporta et al., 1983). The obtained DNA was dissolved in 200 µl of 1X TE buffer and stored at -20°C, and then diluted to 25 ng/µl using double-distilled water to obtain the working solution. DNA quality was checked using 1% agarose gel.

PCR amplification and gel electrophoresis

PCR was performed in 10 µl reaction mixture volume containing 2 µl of DNA, 4.2 µl of ddH_2O, 1 µl of 10X buffer, 1 µl of dNTPs (1 mM), 0.3 µl of $MgCl_2$ (25 mM), 0.5 µl of Taq polymerase, 0.5 µl of the Forward primer (10 mM) and 0.5 µl of the Reverse primer (10 mM). Mineral oil was added to the reaction mixture to prevent evaporation. The PCR reaction was performed using a G-Storm thermal cycler system (384 well alpha-unit). The PCR program used consisted of an initial denaturation at 95°C for 5 min followed by a series of 35 cycles, each of which consisted of a denaturation at 94°C for 4 min, annealing at 55°C for 1 min and extension at 72°C for 2 min. The program ends with a final elongation of 72°C for 7 min. The amplification products were run on an 8% non-denaturing polyacrylamide gel electrophoresis (PAGE) following a modified procedure by AfricaRice biotechnology laboratory in Senegal (Thomson et al., 2010) with 1X TBE buffer at 100 V for 150 min. Three microliters of blue dye were added to the PCR products and 4µl of the final volume were loaded into the gel well with 50 bp ladder. After electrophoresis, the gels were soaked in a solution of Ethidium bromide (3.5%) for 15 min and visualized under UV using Gel Documentation Systems.

SSR markers

Aroma SSR markers

Four markers (RM223, RM210, RM342A, RM152) mapped on chromosome 8 (Temnykh et al., 2000), linked to the region controlling the aroma were used (Table 2).

SSR markers for cooking and eating parameters

Four SSRs including RM170, RM190, RM204, and RM253 located near *Wx* and *Alk* genes were used. These markers were previously mapped to the short arm of chromosome 6 and were reported to be linked to loci controlling rice cooking and eating characteristics (Septiningsih et al., 2003; Aluko et al., 2004; Fan et al., 2005; Shu et al., 2006) (Table 3).

Data analysis

Clearly resolved bands of the genotypes were manually scored using the binary coding system, ‘1’ for presence of band and ‘0’ for absence of band. The resultant matrix was used to calculate genetic similarities among the accessions according to Jaccard’s coefficient (Jaccard, 1908) using NTSYS-pc software package version 2.02e (Rohlf, 2004). Using pairwise similarity matrix of Jaccard’s coefficient, a phylogenetic tree was constructed by the Unweighted Pair-Group Method of Arithmetic average (UPGMA)) module of the NTSYS-pc. Polymorphism information content (PIC) was calculated using the formula:

$$\mathbf{PIC} = \mathbf{1} - \sum_{\mathbf{j=1}}^{\mathbf{n}} \mathbf{P_{ij}}^{\mathbf{2}}$$

where P_{ij} is the frequency of the allele i at locus j. It is calculated for allele. The effective number of alleles was calculated using the formula:

$NE = 1/Pij^2$

The software package SPSS20.0 (SPSS, Chicago, IL, USA) was used for correlation analysis.

RESULTS AND DISCUSSION

Overall allelic diversity

All the SSR markers used in this study were polymorphic.

Table 1. Origin and physico-chemical properties of studied varieties.

Varieties	Parents	Origin	AC (%)	ASV	GT	Aroma
Gambiaka Bénin	-	Benin	-	-		No Aroma
Gambiaka Burkina Faso	-	Burkina Faso	-	-		No Aroma
Gambiaka KK	-	Malia	24.70	5,2	Intermediary	No Aroma
Gambiaka Nigeria	-	Nigeria	-	-		No Aroma
Basmati Punjab	-	India	21.24	7	Low	Aroma
Basmati 270	-	India	-	-		Aroma
Basmati 370	-	India	22.80	4.2	Intermediary	Aroma
Dom Seophid	-	Iran	19.9	3.5	Intermediary	Aroma
WAB 638-1	DR2/ DR2	WARDA (Ivory Coast)	24	2.3	High	Aroma
KDM 105	-	Thaïlande	15	6.8	Low	Aroma
TS 2	-	Taiwan	22	5	Intermediary	Not Aroma
Sahel 108	IR30(BHP)/BABAWE//IR 36	IRRI (Philippines)	27	2	High	No Aroma
Sahel 134	IR 1791-5-4-3-3/IR 9129-209-2-2-2-1	IRRI (Philippines)	25	2	High	No Aroma
Sahel 159	IR 13240-108-2-2-3/IR 9129-209-2-2-2-1	IRRI (Philippines)	24	2	High	No Aroma
Sahel 177	Sahel 134/IR66231-37-1-2	AfricaRice (Saint Louis)	30	2	High	Aroma
Sahel 201	IR 2071-586/ BG 400-1	Sri Lanka	28.10	6	Low	No Aroma
Sahel 202	TOX 494-3696/TOX 711/BG6812	Nigeria	27.70	2	High	No Aroma
Sahel 208	ITA 212/ UPL RI 7	ITA (Nigeria)	29	3	Intermediary	No Aroma
Sahel 209	TSY/MOROBERKAN//ITA306	ITA (Nigeria)	28	5	Intermediary	No Aroma
Sahel 210	-	Latin America	20	2	High	No Aroma
Sahel 217	Sahel 201/ 4456	AfricaRice (Saint Louis)	30.90	4	Intermediary	No Aroma
Sahel 222	Sahel 201/ 4456	AfricaRice (Saint Louis)	31.90	3	Intermediary	No Aroma
Sahel 305	IR64/4456	AfricaRice (Saint Louis)	27.80	7	Low	No Aroma
Sahel 317	IR64/4456	AfricaRice (Saint Louis)	27.25	7	Low	No Aroma
Sahel 328	Sahel 134/ IR66231-37-1-2	AfricaRice (Saint Louis)	31.50	7	Low	Aroma
Sahel 329	Jaya/Basmati 370	AfricaRice (Saint Louis)	30.90	7	Low	Aroma
Nerica-S-19	Tog5681/2*IR64/IR31785	AfricaRice (Saint Louis)	32	2	High	No Aroma
Nerica-S-21	Tog5681/2*IR64/IR31785	AfricaRice (Saint Louis)	32	4	Intermediary	No Aroma
Nerica-S-36	Tog5681/2*IR1529//IR1529	AfricaRice (Saint Louis)	31.60	2	High	No Aroma
Nerica-S-44	IR64/ Tog5681/4*IR64	AfricaRice (Saint Louis)	28.80	3	Intermediary	No Aroma

References of quality traits data (ASV, AC, Aroma) (Traore et al., 2015; Tabkhkar et al., 2012).

The eight markers produced 45 alleles with an average of 5.63 alleles per locus among the 30 rice genotypes. The number of alleles per primer ranged from three for RM204 to eight for RM190 and RM342A (Table 4). This number is similar to the average value of 5.89 alleles per locus obtained in a study of Philippian rice cultivars using a set of 151 polymorphic SSR markers (Lapitan et al., 2007). But it is higher than the

Table 2. SSR markers used for aroma in this study

Markers	Chromosome	Repeat types		References
RM152	8	Fwd. Rev.	GAAACCACCACACCTCACCG CCGTAGACCTTCTTGAAGTAG	Temnykh et al. (2000)
RM210	8	Fwd. Rev.	TCACA T TCGGTGGCATTG CGAGGATGGTTGTTCACTTG	Temnykh et al. (2000)
RM223	8	Fwd. Rev.	GAGTGAGCTTGGGCTGAAAC GAAGGCAAGTCTTGGCACTG	Temnykh et al. (2000)
RM342A	8	Fwd. Rev.	CCATCCTCCTACTTCAATGAAG ACTATGCAGTGGTGTCACCC	Temnykh et al. (2000)

Table 3. SSR linked to QTLs detected on chromosome 6 responsible of eating and cooking properties of rice (Tabkhkar et al. 2012).

Marker	Sequence	Studied traits	QTLs	Parents	References
RM 170	Fwd. TCGCGCTTCTTCCTCGTCGACG Rev. CCCGCTTGCCGTTCATCCCTCC	AC	*ac6a*	Zhenshan97/H94	Fan et al. (2005)
		GC	-	IR64/IRGC105491	Septiningsih et al. (2003)
RM 190	Fwd. CTTTGTCTATCTCAAGACAC Rev. TTGCAGATGTTCTTCCTGATG	AC	*ac6a*	Zhenshan97/H94	Fan et al. (2005)
		GC	*gc6b*	Zhenshan97/H94	Fan et al. (2005)
		GT	*asv6a*	Zhenshan97/H94	Fan et al. (2005)
		AC	*amy6*	Caiapo/IRGC103544	Aluko et al. (2004)
		GT	*Alk 6-1*	Caiapo/IRGC103544	Aluko et al. (2004)
RM 204	Fwd. GTGACTGACTTGGTCATAGGG Rev. GCTAGCCATGCTCTCGTACC	GT	-	-	He et al. (1999)
RM 253	Fwd. TCCTTCAAGAGTGCAAAACC Rev. GCATTGTCATGTCGAAGCC	AC	*ac6b*	Zhenshan97/H94	Fan et al. (2005)
		GC	*gc6b*	Zhenshan97/H94	Fan et al. (2005)
		GT	*asv6b*	Zhenshan97/H94	Fan et al. (2005)
		AC	-	IR64/IRGC105491	Septiningsih et al. (2003)
		GC	-	IR64/IRGC105491	Septiningsih et al. (2003)
		GT	-	Huangyu B/II32 B	Shu et al. (2006)
		GT	*alk6-2*	Caiapo/IRGC103544	Aluko et al. (2004)

Table 4. Characteristics of SSR markers in all the studied genotypes.

Markers	Chromosome	Studied Traits	Motifs	Number of alleles	Number of effective alleles	PIC
RM170	6	AC, GC	(CCT)7	4	3.80	0.74
RM190	6	AC, GC, GT	(CT)11	8	4.62	0.78
RM204	6	GT	(CT)44	3	1.63	0.39
RM253	6	AC, GC, GT	(GA)25	6	4.35	0.77
RM152	8	Aroma	(GGC)10	4	2.15	0.53
RM210	8	Aroma	(CT)23	5	3.15	0.67
RM223	8	Aroma	(CT)25	7	3.79	0.74
RM342A	8	Aroma	(CAT)12	8	6.29	0.84

average value of 4.3 alleles per locus ranging from 2 to 9 alleles reported in a Venezuelan rice cultivars genetic diversity assessment (Herrera et al., 2008) and the value of 3.33 alleles across 9 polymorphic loci obtained using a set of 24 SSR markers for the characterization and discrimination of 12 elite aromatic rice genotypes (Sajib et al., 2012). This study average alleles number is also higher than the value (3.13) reported in a study using a set of 8 markers linked to aroma and cooked kernel elongation covering chromosomes 3, 4, 8 and 9 to assess the genetic diversity among thirteen rice varieties from Kenya and Tanzania (Kioko et al., 2015). Although the number of polymorphic SSR markers in Lapitan et al. (2007) was higher than the number used in this study, almost similar average values were noticed, this can be a reflection of a high level of diversity among our cultivars. However, the allele's average number reported in this study was lower than the value of 9.3 reported in a study using a larger scale of accessions (238) representing both *japonica* and *indica* cultivated rice (Yang et al., 1994) or the value of 7.8 reported in a genetic analysis of 69 Indian aromatic rice cultivars using 30 fluorescently labeled rice SSR markers (Jain et al., 2004). Furthermore, this study's average number is less than the value of 6.33 alleles per locus recorded in a DNA fingerprinting study of 34 rice genotypes using a small set of three SSR marker for a diversity (Rahman et al., 2009). This difference observed with those reports might be due to the use of diverse germplasm and higher number of rice accessions used in the aforementioned studies. The SSR markers linked to aroma produced 26 alleles with an average of six loci per marker, which is slightly higher than the average number (5.25) produced by the marker linked to QTLs controlling cooking and eating quality. The average number of alleles linked to aroma (6) is higher than the average value of 4.7 alleles per locus reported in a Basmati rice genetic diversity analysis using 26 SSR marker associated with aroma and cooked kernel elongation (Jain et al., 2006). The average value of SSR markers linked to the cooking and eating quality is almost similar to the value of 5.86 reported in a genetic diversity assessment of 48 rice cultivars using markers linked to the cooking and eating quality of rice (Tabkhkar et al., 2012).

Rare alleles were also observed in this study. Alleles observed in less than 5% of all the rice varieties (commonly termed as rare) were investigated and a total of four (8.9% of the total number of alleles) were identified at three loci RM190 (2), RM253 (1) and RM223 (1). Gambiaka Burkina Faso, Nerica-S-36, Sahel 159 and Sahel 329 had each one rare allele. RM190 revealed the highest number of rare allele and might be useful in the creation of fingerprints of the varieties used in this study. The effective number of alleles varied from 1.63 for RM204 to 6.29 for RM342A with an average of 3.72 which was very much similar to the value of 3.74 recorded by Tabkhkar et al. (2012) and higher than the 2.19 reported by Kibria et al. (2009).

PIC value

The varying PIC values generated by the markers reflect the discriminating power of a particular marker by taking into account the number of alleles at each locus and their relative frequencies among the tested varieties. In our study, PIC values varied from 0.39 (RM204) to 0.84 for RM342 with an overall average of 0.68. The highest PIC value for markers linked to QTLs controlling cooking and eating quality is recorded for RM190 (0.78). The overall average value is similar to the PIC value reported by Lapitan et al. (2007) and the one reported by Jain et al. (2004), but it is higher than the value reported by Kioko et al. (2015) and the one recorded by Sajib et al. (2012). There is no correlation between the repeat number of SSR motifs and the PIC value and between the repeat number of the SSR motifs and the number of alleles. The later observed pattern was consistent with Kioko et al. (2015) report but not in concordance with the results of Herrera et al. (2008) who found that the maximum number of repeats within the SSRs was significantly correlated with the number of alleles at a locus (r = 0.505, $P < 0.01$). However, a significant correlation was observed between the PIC value and the number of detected alleles (r=0.816, p-value less than 0.05): the higher the allele number, the higher the observed value

Table 5. Pairwise genetic similarities obtained among the 30 rice varieties using eight SSR markers.

Parameter	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
V2	0.85													
V3	0.85	1												
V4	0.70	0.86	0.86											
V5	0.54	0.62	0.62	0.60										
V6	0.55	0.56	0.56	0.56	0.95									
V7	0.59	0.60	0.60	0.52	0.74	0.73								
V8	0.61	0.68	0.68	0.63	0.91	0.86	0.82							
V9	0.07	0.20	0.20	0.19	0.23	0.20	0.21	0.24						
V10	0.09	0.20	0.20	0.19	0.24	0.21	0.21	0.27	0.94					
V11	0.07	0.20	0.20	0.23	0.24	0.21	0.21	0.29	0.94	1				
V12	0.19	0.27	0.27	0.27	0.27	0.33	0.33	0.31	0.59	0.62	0.58			
V13	0.24	0.33	0.33	0.32	0.29	0.36	0.36	0.33	0.43	0.44	0.39	0.71		
V14	0.30	0.42	0.42	0.37	0.42	0.45	0.45	0.47	0.65	0.63	0.73	0.63	0.71	
V15	0.50	0.52	0.52	0.42	0.58	0.59	0.57	0.65	0.19	0.23	0.23	0.36	0.63	0.52

of the PIC (Table 5). The same observation was also made by Lapitan et al. (2007) and in a genetic diversity study of 193 parental lines of different origins using 101 well-distributed SSR markers (Yu et al., 2003). The level of polymorphism observed in this study is relatively high and can be explained by the presence in our studies of varieties from different origins.

Cluster analysis and genetic relationships

Genetic similarity based on Jaccard coefficient off similarity implemented in the NTSYS-pc software ver.2.02e was used to assess the level of relatedness among the studied cultivars (Table 6). The pairwise genetic similarity ranged from 0.07 between the traditional aromatic rice Basmati 370 and the landrace Gambiaka Nigeria widely spread in Nigeria to a maximum similarity of 1 between Gambiaka Burkina Faso and Gambiaka Kokoum, Basmati 370 and Basmati 270, Sahel 108 bred in IRRI (Philippines) and Sahel 208 bred in ITA (Nigeria), Sahel 208 and Sahel 201 bred in Sri Lanka, Sahel 108 and Sahel 201, Sahel 209 and Sahel 202 both bred in ITA, as well as Sahel 305 and Sahel 317. Most of the Sahel varieties showing maximum similarity coefficient of 1 have the same AC, but differ in term of GT except Sahel 317 and Sahel 305, two varieties derived from the same breeding program and having the same AC and GT. This result was expected giving that among the four markers used for the cooking and eating parameters, only one (RM204) was reported specifically linked to QTLs controlling GT. Therefore, more markers linked to Loci controlling GT is needed for a more precise discrimination of the varieties used in this study. A high coefficient of similarity was observed within the two groups of traditional varieties present in our study. Within the Gambiaka group, the similarity coefficient varied from 0.70 between Gambiaka Benin and Gambiaka Nigeria to a maximum similarity of 1 between Gambiaka Kokoum and GambiakaBurkina Faso. In the Basmati group, the similarity coefficient varied from 0.94 between Basmati Punjab and the two others Basmati varieties (Basmati 370 and Basmati 270) to a maximum similarity of one between Basmati 270 and Basmati 3710. This reflects an intra-group homogeneity inside these two traditional groups. Furthermore, the coefficient of similarity is low between the Basmati varieties and the other groups. For example, the maximum coefficient of similarity value observed between the Basmati group and the interspecific NERICA (value observed between Nerica-s-44 and Basmati 370) was 0.29 and a maximum value of 0.23 was observed between the Gambiaka and the Basmati group. This observation confirmed that the traditional Basmati varieties have an intra-group homogeneity and are different from *indica* and interspecific types (Jain et al., 2004; Das et al., 2013). In the case of the improved varieties Sahel, the maximum values of the similarity coefficient with Basmati rice was observed with the three bred aromatic varieties Sahel 177, Sahel 328 and Sahel 329. The coefficient was 0.29 between Sahel 329 and Basmati 370, which is one of the parents used in the Sahel 329 breeding program parent. As expected, observed genetic similarity between aromatic varieties were high and improved varieties having the same AC have a high level of similarity.

Genetic similarity values obtained among the cultivars led to the construction of an UPGMA-based dendrogram as shown in Figure 1. At 63% level of similarity, the UPGMA diagram showed five major groups. Group 1 corresponded to two aromatic varieties; the Iranian traditional aromatic variety Domseophid and the variety Wab 638-1 created by mass selection at the ADRAO

Table 6. Pairwise genetic similarities obtained among the 30 rice varieties using eight SSR markers.

Parameter	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28	V29
V2	0.85																												
V3	0.85	1																											
V4	0.70	0.86	0.86																										
V5	0.54	0.62	0.62	0.60																									
V6	0.55	0.56	0.56	0.56	0.95																								
V7	0.59	0.60	0.60	0.52	0.74	0.73																							
V8	0.61	0.68	0.68	0.63	0.91	0.86	0.82																						
V9	0.07	0.20	0.20	0.19	0.23	0.20	0.21	0.24																					
V10	0.09	0.20	0.20	0.19	0.24	0.21	0.21	0.27	0.94																				
V11	0.07	0.20	0.20	0.23	0.24	0.21	0.21	0.29	0.94	1																			
V12	0.19	0.27	0.27	0.27	0.27	0.33	0.33	0.31	0.59	0.62	0.58																		
V13	0.24	0.33	0.33	0.32	0.29	0.36	0.36	0.33	0.43	0.44	0.39	0.71																	
V14	0.30	0.42	0.42	0.37	0.42	0.45	0.45	0.47	0.65	0.63	0.73	0.63	0.71																
V15	0.50	0.52	0.52	0.42	0.58	0.59	0.57	0.65	0.19	0.23	0.23	0.36	0.63	0.52															
V16	0.58	0.55	0.55	0.46	0.75	0.79	0.65	0.70	0.14	0.13	0.12	0.21	0.26	0.37	0.67														
V17	0.59	0.56	0.56	0.44	0.70	0.71	0.61	0.70	0.14	0.15	0.14	0.26	0.27	0.37	0.67	0.94													
V18	0.44	0.43	0.43	0.37	0.60	0.61	0.46	0.60	0.17	0.21	0.21	0.25	0.31	0.40	0.71	0.68	0.68												
V19	0.48	0.56	0.56	0.46	0.56	0.56	0.48	0.56	0.30	0.27	0.29	0.35	0.38	0.52	0.52	0.65	0.63	0.48											
V20	0.56	0.52	0.52	0.43	0.74	0.78	0.63	0.68	0.11	0.10	0.08	0.19	0.23	0.35	0.65	1	0.93	0.67	0.63										
V21	0.59	0.56	0.56	0.44	0.77	0.80	0.68	0.77	0.18	0.18	0.18	0.26	0.27	0.41	0.67	0.94	0.89	0.68	0.63	0.93									
V22	0.60	0.57	0.57	0.43	0.71	0.74	0.62	0.71	0.11	0.13	0.11	0.20	0.25	0.36	0.68	1	0.94	0.70	0.64	1	0.94								
V23	0.59	0.56	0.56	0.44	0.77	0.80	0.68	0.77	0.18	0.18	0.18	0.26	0.27	0.41	0.67	0.94	0.89	0.68	0.63	0.93	1	0.94							
V24	0.50	0.42	0.42	0.31	0.61	0.62	0.59	0.61	0.15	0.16	0.15	0.32	0.35	0.39	0.74	0.71	0.70	0.75	0.48	0.69	0.79	0.72	0.79						
V25	0.57	0.60	0.60	0.48	0.74	0.76	0.65	0.74	0.21	0.21	0.21	0.29	0.31	0.45	0.64	0.88	0.85	0.65	0.67	0.88	0.95	0.89	0.95	0.75					
V26	0.62	0.58	0.58	0.46	0.65	0.67	0.64	0.65	0.14	0.15	0.14	0.27	0.33	0.38	0.62	0.88	0.84	0.64	0.65	0.87	0.84	0.88	0.84	0.74	0.89				
V27	0.65	0.68	0.68	0.55	0.73	0.75	0.64	0.73	0.19	0.19	0.19	0.28	0.35	0.44	0.70	0.88	0.84	0.64	0.76	0.87	0.84	0.88	0.84	0.65	0.84	0.83			
V28	0.62	0.70	0.70	0.57	0.70	0.71	0.61	0.70	0.22	0.22	0.22	0.30	0.38	0.46	0.67	0.82	0.80	0.61	0.77	0.81	0.80	0.83	0.80	0.62	0.85	0.84	1		
V29	0.48	0.56	0.56	0.46	0.56	0.56	0.48	0.56	0.30	0.27	0.29	0.35	0.38	0.52	0.52	0.65	0.63	0.68	0.68	0.63	0.63	0.64	0.63	0.48	0.67	0.65	0.76	0.77	
V30	0.36	0.35	0.35	0.30	0.50	0.54	0.48	0.50	0.29	0.27	0.29	0.35	0.33	0.52	0.52	0.62	0.56	0.54	0.62	0.60	0.63	0.57	0.63	0.61	0.60	0.52	0.52	0.50	0.91

V1=Gambiaka Nigeria, V2=Gambiaka Kokoum, V3=Gambiaka Burkina Faso, V4=Gambaiaka Benin, V5=Nerica-S-19, V6=Nerica-S-21, V7=Nerica-S-36, V8=Nerica-S-44, V9=Basmati Punjab, V10=Basmati 270, V11=Basmati 370, V12=Domseophid, V13=Wab638-1, V14=KDM105, V15=TS2, V16=Sahel 108, V17= Sahel 134, V18= Sahel 159, V19=Sahel 177, V20=Sahel 201, V21=Sahel 202, V22=Sahel 208, V23=Sahel 209, V24=Sahel 210, V25=Sahel 217, V26=Sahel 222, V27=Sahel 305, V28=Sahel 317, V29=Sahel 328, V30=Sahel 329.

(former name of AfricaRice) in Bouake (Ivory Coast). The two varieties showed a 71% Level of similarity. Group 2 consisted of four traditional aromatic varieties: the three Basmati rice and KDM 105 originated from Thailand. As expected, the tree Basmati formed a sub-cluster (2B) separated from KDM 105 which formed a sub-cluster of its own (v2A). This is explained by the homogeneity of the basmati group mentioned in previous paragraph. Group 3 consisted of the three improved aromatic varieties; Sahel 177, Sahel 328 and Sahel 329 all bred at Africa Rice (Saint-Louis) and released in Senegal. This group could be further divided at about 66% level of similarity in two sub-groups 3A and 3B. Sub-group 3A contained the two aromatic lines Sahel 328 (AC=31.50, ASV=7) and Sahel 329 (AC=30.90, ASV =7) having both a high AC associated with a

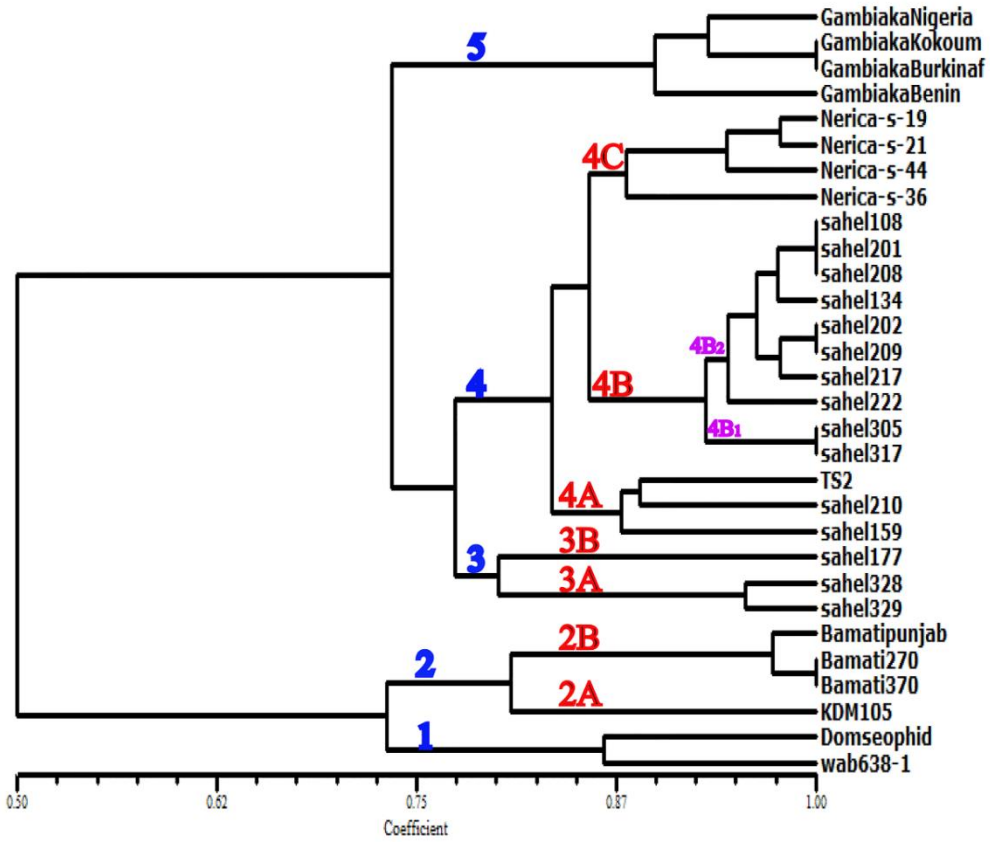

Figure 1. Dendrogram derived from UPGMA cluster analysis baed on Jaccard similarity coefficient showing genetic diversity and relatedness among the 30 rice varieties.

low GTre, while sub-group 3B only contained Sahel 177 having a high AC associated with a high GT (AC=30 and GT=2). Group 4 was the most diversified group and clustered 17 out of 30 varieties. This group could be separated into three sub-groups based on the origin and the cooking and eating characteristics of the varieties. The first subs-group 4A at about 70% level of similarity contained three varieties TS2 (AC=22, ASV=5), Sahel 159 (AC=24, ASV=2) and Sahel 210 (AC=20, ASV=2) with intermediate AC and high GT except TS2 which has an intermediate GT. Sahel 210 originated from Latin American and is released in Senegal, while TS2 originated from Taiwan is released in Burkina Faso. The sub-group 4C at a similarity level of about 75% is comprised of the four interspecific NERICA which have a high content of amylose. The last sub-group 4B contained 10 Sahel varieties and can be further divided in two small clusters $4B_1$ and $4B_2$. Cluster $4B_1$ contained two sister lines; Sahel 305 (AC = 27.80; ASV= 7) and Sahel 317 (AC = 27.25; ASV=7) which have a high AC associated with a low GT. The second small cluster gathered the remaining ten Sahel varieties. All the varieties present in that group have in common a high level of AC but belong to different classes of GT (from low to high) and are from different origins. The high GT class contained Sahel 108 (AC=27, ASV=2), Sahel 134 (AC=25, ASV=2), Sahel 202 (AC=27.70, ASV=2), and Sahel 208 (AC = 29, ASV= 3). This group contained varieties released in some African countries (Sahel 208 from bred at International Institute for Tropical Agriculture in Nigeria is released in Senegal, while Sahel 208 bred originated from Nigeria is only released in Senegal. Sahel 209 (AC = 28; ASV= 5) released in Senegal and bred at the International Institute for Tropical Agriculture (IITA, Nigeria), Sahel 217 (AC = 30.90; ASV= 4) and Sahel 222 (AC= 31.90; ASV= 5) bred at AfricaRice (Saint-Louis, Senegal) belong to intermediary GT class, while Sahel 201 (AC = 28.10; ASV=6) originated from SriLanka and released in Senegal, belong to the low GT class. Group 5 corresponded to the traditional varieties Gambiaka group with high AC and high ASV (low GT) well spread in West Africa countries.

Conclusion

The present study provides an insight on the genetic diversity of the rice genome regions controlling aroma,

cooking and eating parameters of some rice varieties commonly grown in Africa. Aroma, cooking and eating quality are the main quality traits that determine the rice market value in Africa and around the world. The results provided by SSR markers can be useful for efficient breeding programs since we were able to group the varieties based on their AC and aroma. However, the discrimination was not accurate when it comes to the GT of the varieties underlining the fact that more markers linked to the GT are required for a more precise discrimination. RM 342 has the highest PIC value and can be used to differentiate aromatic varieties from non-aromatic ones; it is followed by RM 190, which was very informative on the cooking and eating parameters of our varieties. Based on the clustering data, different breeding programs can be designed using genetically distant varieties in order to produce varieties carrying aroma and interesting cooking and eating quality. For example, an interspecific cross can be made between the varieties of the traditional aromatic group and the landraces Gambiaka or between the two groups and the improved Sahel varieties in order to get hybrids carrying more interesting cooking and eating properties and aroma. These results confirmed also the powerfulness of SSR markers to assess the genetic diversity of different cultivars reported by previous studies and strengthen the fact that SSR markers could be used to save time during the characterization of breeding materials at AfricaRice center. However, more markers linked to the cooking and eating properties or covering can be used for further studies in order to come up with more conclusive results for the fingerprinting and the discrimination of varieties used in this study based on physico-chemical parameters.

Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

RM, Rice microsatellite; **SSR,** simple sequence repeats; **UPGMA,** unweighted pair group method with arithmetic averages.

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