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The use of multiplexed simple sequence repeat (SSR) markers for analysis of genetic diversity in African rice genotypes

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Rice is an emerging food and cash crop in Eastern Africa. Thousands of germplasm accessions have been introduced from major rice breeding centers, such as the International Rice Research Institute (IRRI), and Africa Rice but the genetic variability among the introduced rice germplasm is unknown. Knowledge on genetic diversity would be useful in designing measures for comprehensive breeding and conservation. To address this knowledge gap, 10 highly polymorphic rice simple sequence repeat (SSR) markers were used to characterize 99 rice genotypes to determine their diversity and place them in their different population groups. The SSR markers were multiplexed in 3 panels to increase their throughput. An average of 15.9 alleles was detected, ranging from 6 alleles detected by marker RM7 to 30 by marker RM333. The UPGMA dendogram based on Nei's genetic distance cluster analysis, revealed 5 genetic groups among the genotypes tested. Analysis of molecular variance indicated that 97% of the diversity observed was explained by differences in the genotypes themselves, and only 3% was due to the sources from which the genotypes were obtained. This study sets the stage for further diversity analysis of all the available germplasm lines using SSR markers to ensure effective utilization and conservation of the germplasm.

Key words: Genetic diversity, simple sequences repeat (SSR) markers, multiplexing, rice genotypes, structure.

INTRODUCTION

Rice is an important food and commercial crop in Africa but in a country like Uganda, domestic consumption is higher than production (FAOSTAT, 2012). Uganda's rice cultivars (*Oryza sativa*, 2n = 24, AA) include NERICA lines, landraces, and varieties developed by the Cereals Program of Uganda's National Crops Resources Research Institute (NaCRRI). In an effort to identify the best and most diverse candidates with resistance to local stresses that may provide rapid genetic improvement and be incorporated into Uganda's national rice breeding efforts,

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NaCRRI breeders have imported thousands of rice lines from the International Rice Research Institute (IRRI) and AfricaRice center and evaluating them alongside locally-adapted lines and breeding materials. These available germplasm lines are morphologically diverse but lack adequate documented information on their genetic potential and diversity. This deters their apt utilization as a potential source of desired genes and their effective conservation for future use. Constraints to rice production in Uganda include both biotic and abiotic stresses, and these are frequently evolving. This requires genetically diverse materials to check genetic erosion resulting from the continued adoption of only particular varieties and thereby maintain and/or increase the region's rice production.

Genetic diversity in rice germplasm can be assessed by both observed morphological traits and molecular markers (Chakravarthi and Naravaneni, 2006). Though morphological traits have been used as markers for assessing genetic diversity in the past, they are often influenced by the environment, limited in number and are therefore unreliable in themselves (Miller et al., 1989). Different types of genetic (DNA) markers are available nowadays including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), single nucleotide polymorphisms (SNP), simple sequence repeats (SSR) among others. Each marker type deferrers in cost, time requirement, degree of polymorphism detected, application and principle. SSRs are the preferred markers for genetic analysis in rice and have been used for a number of studies (Akagi et al., 1997) because of their abundance and well distribution throughout the genome. As genetic markers, they are co-dominant, detect high levels of allelic variation, and can be multiplexed to increase the throughput (Guichoux et al., 2011). They are also technically efficient, cost-effective and able to analyze both *indica* and *japonica* rice groups (McCouch et al., 2002).

Morishima and Oka (1981) divided the cultivated species of rice into two groups; *indica* and *japonica*. The domestication process was believed to have caused the difference between these two groups, including their reproductive barriers (Harushima et al., 2002). Furthermore, three morphological groups were described by ecological distribution; tropical *japonica*, temperate *japonica*, and *indica* (Glaszmannand Arraudeau, 1986). McCouch et al. (2002) developed a high-density rice genetic map from fully sequenced BAC (Bacterial Artificial Chromosome) and PAC (P1-derived Artificial Chromosome) clones representing 83% of the total rice genome BAC (Bacterial Artificial Chromosome) and PAC (P1-derived Artificial Chromosome). These consist of 2340 validated markers and the information was integrated into gramene, a comparative grass genome database (http://archive.gramene.org/markers/microsat/ssr.html) to increase the density and utility of the SSR map in rice (McCouch et al., 2002).

In this study, the utility of this vast genetic resource was evaluated by identifying SSR markers reliable for use in rapid molecular characterization of the collection of rice genotypes available in NaCRRI. As a pilot project in the program, only one marker was picked per rice chromosome basing on its degree of polymorphism as reported by other rice researchers (Drame et al., 2011; Chakravarthi and Naravaneni, 2006; Ni et al., 2002). This was aimed at detecting genetically diverse lines and classification into their different groups.

MATERIALS AND METHODS

Plant material

Ninety nine rice genotypes (Table 1) were used in this study. They included the local varieties; Supa, Kaiso, Sindano, NERICA 1, NERICA 2 and NERICA 10, and introductions from IRRI and AfricaRice, interspecific and intraspecific breeding lines from NaCRRI Cereals Program, and collections from Ugandan farmers. The study lines were selected on the basis of their phenotypic diversity observed in the field. They were planted in NaCRRI in central Uganda (located at 000 32' N latitude and 320 53' E longitude, and an altitude of 1,150 m asl). After one month of establishment, fresh young leaves were harvested from each genotype for DNA analysis.

DNA extraction and quantification

The genomic DNA was extracted from about 100 mg of frozen leaf tissue at the Forestry and Agricultural Biotechnology Institute (FABI) of the University of Pretoria, South Africa using the Qiagen DNeasy plant kit (QIAGEN, 2006). Aliquots of 10 µl of the freshly extracted DNA were stained with Syber green and electrophoresed on 1% agarose gel then visualized under a UV transilluminator (BioRad) to assess their quality. The concentration of DNA in the samples was determined using a Nanodrop D-1000 spectrophotometer, and then diluted to 5 ng/µl prior to use (QIAGEN, 2006).

Polymerase chain reaction (PCR)

The diluted DNA samples were amplified using 14 SSR markers (RM1, RM154, RM7, RM261, RM249, RM3, RM125, RM223, RM316, RM333, RM206, RM20A, RM273 and RM252) selected on the basis of their polymorphism level reported in rice by Chakravarthi et al. (2006) and Drame et al. (2011). The sequences of these molecular markers were obtained from the Gramene website (www.gramene.org/microsat/2013). The primers were synthesized by Inqaba Biotechnologies Inc (Pretoria, South Africa). PCR was done to confirm amplification and polymorphism of the markers in ten randomly selected DNA samples prior to labeling with fluorescent dyes. The PCR amplifications were carried out separately for each marker, in a 96-well DNA Engine Peltier Thermal Cycler (Biorad). The total volume of 10 µl PCR mix was constituted by 5 ng/µl DNA, 1x PCR buffer (Fermentas: 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgSO_4$, 1.5 mM $MgCl_2$), 2 mM dNTPs mix, 0.2 µM each of the forward and reverse primers, 25 mM $MgCl_2$, and 0.5U Taq polymerase (Fermentas). The PCR program used was 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. A final extension step at 72°C for 5 min was included. The markers such as RM1, RM261, RM3 and RM316 which were not as polymorphic as expected were not labelled.

Table 1. List of 99 genotypes used in the study and their alleles detected with 10 markers.

No.	Genotype	Allele										
		Origin	RM125 / Chr7	RM249 / Chr5	RM7 / Chr3	RM206 / Chr11	RM252 / Chr4	RM273 / Chr4	RM154 / Chr2	RM20A / Chr12	RM223 / Chr8	RM333 / Chr10
1	NERICA-L28	AfricaRice	122/122	121/121	182/182	166/166	243/243	210/210	168/168	231/257	157/157	174/177
2	NERICA 1 X WAC 116	NaCRRI	125/125	121/121	174/174	168/178	251/253	200/200	165/148	210/231	157/157	185/189
3	BGM 111	IRRI	125/125	117/117	168/168	184/184	195/195	200/200	189/194	228/231	147/157	177/177
4	FAROX 503-3-11-F43-2-1	AfricaRice	125/125	119/119	168/168	132/132	195/195	200/204	181/201	210/228	157/157	185/189
5	MET 121	AfricaRice	125/125	119/119	182/182	132/132	195/195	200/204	194/194	210/210	157/157	189/192
6	K5 X NERICA 1	NaCRRI	122/122	117/121	182/182	132/132	195/195	200/200	176/194	225/228	157/157	189/191
7	NERICA 1 X NERICA 4	NaCRRI	125/125	119/119	174/174	132/138	195/195	204/204	148/201	210//311	155/157	185/189
8	IR-80353-63-B-38-1-2-B	IRRI	122/125	119/119	182/182	null	null	null	181/201	228/228	157/157	174/174
9	IR-77372-7-B-4-1-1-B	IRRI	122/125	119/119	174/174	132/132	195/195	204/204	201/201	210/311	157/157	189/189
10	IR-80352-4-B-24-2-3-B	IRRI	125/125	119/119	174/174	190/192	195/195	202/202	148/203	210/210	147/155	155/155
11	IR 83372-B-B-133-2	IRRI	148/148	117/121	182/182	168/168	195/195	200/200	148/208	210/231	147/157	177/177
12	NM7-8-2-B-P-2-1	NaCRRI	125/125	145/145	168/174	132/132	249/251	200/200	181/201	210/276	157/157	185/189
13	WAB 2099-WAC1-TGR5-B	AfricaRice	122/122	119/119	182/182	153/153	257/259	200/200	144/158	212/212	155/158	124/193
14	WAB 2060-3-FKR2-3-TGR5-B	AfricaRice	125/125	119/123	174/174	190/192	195/195	200/200	155/194	210/239	155/155	185/189
15	BGM-5	IRRI	148/148	121/121	182/182	170/170	247/249	210/210	148/189	216/257	157/157	183/183
16	NERICA 4 X NERICA 6	NaCRRI	125/125	119/121	182/182	132/132	195/195	200/200	148/194	210/210	147/147	189/192
17	NERICA 4 X K5	NaCRRI	122/125	119/121	182/182	132/146	195/195	204/204	148/201	210/257	157/157	189/189
18	WAB 2151-TGR2-WAT B3	AfricaRice	122/122	121/121	182/182	132/132	195/195	204/210	null	210/257	157/157	220/248
19	Sindano	Farmers	125/125	121/121	176/176	199/199	195/195	202/202	157/183	210/311	157/157	212/209
20	AER-41	IRRI	148/148	121/121	182/182	168/168	247/249	210/210	176/176	231/260	155/155	185/189
21	NERICA 6 X NERICA 4	NaCRRI	125/125	119/123	174/174	190/192	195/195	200/200	181/194	210/228	155/157	174/189
22	ART3-11L1P1-B-B-2	IRRI	125/125	119/119	174/174	132/132	195/195	204/204	201/201	210/210	157/157	189/189
23	IR 84852-B-44-1AER	IRRI	122/122	119/119	168/168	146/146	247/249	210/210	157/157	216/216	157/157	147/177
24	Namche-5	NaCRRI	122/122	119/119	182/182	105/151	257/259	200/200	181/181	216/216	157/157	157/181
25	WAC 117	AfricaRice	148/148	121/121	168/168	168/168	251/253	200/200	189/189	231/276	157/157	177/177
26	NERICA 4 X WAC 116	NaCRRI	125/125	121/121	174/174	132/168	195/195	204/204	157/157	210/216	157/157	177/179
27	NERICA 1 X GIGANTE	NaCRRI	122/125	119/119	174/174	190/192	195/195	204/204	147/196	210/258	147/147	187/189
28	WAC 117 X NERICA 4	NaCRRI	null	null	null	132/132	251/253	200/204	157/189	231/276	157/157	177/177
29	NERICA 1 X NERICA 6	NaCRRI	122/125	119/119	182/182	151/151	195/195	204/204	157/181	210/257	157/157	155/177
30	IR-80352-4-B-24-2-3-B	IRRI	122/122	115/115	170/170	146/146	217/217	210/210	165/201	234/257	138/157	174/177
31	NM7-5-2-B-P-79-7	NaCRRI	125/125	123/123	174/174	194/195	195/195	204/204	194/196	210/210	161/161	194/196
32	WAC 116	AfricaRice	116/122	121/121	182/182	146/146	217/217	210/210	157/189	231/257	157/157	174/177
33	AER-75	IRRI	144/148	121/121	168/168	168/168	251/253	200/200	189/189	231/276	165/165	174/177
34	NERICA 1 X NERICA 6	NaCRRI	122/122	119/119	182/182	151/151	257/259	200/200	181/181	210/228	157/157	177/177
35	BGM-78	IRRI	122/122	121/121	182/182	146/146	217/217	210/210	138/201	234/254	138/138	174/177
36	MET 124	AfricaRice	125/125	117/117	174/174	146/146	259/261	200/200	176/176	234/257	157/157	183/183
37	NM7-27-1-B-P-77-6	NaCRRI	122/122	121/121	182/182	178/178	195/195	202/202	147/196	210/304	147/147	185/189
38	NERICA 1 X K85	NaCRRI	122/122	121/121	182/182	168/168	217/217	210/210	147/176	231/260	157/157	174/177
39	NM7-29-4-B-P-80-8	NaCRRI	125/125	123/123	174/174	182/182	195/195	204/204	196/196	210/277	161/161	182/185

Table 1. Contd.

40	AER-41	IRRI	122/122	119/119	170/170	146/146	247/249	210/210	148/201	216/257	157/157	177/177
41	WAB95-B-B-40-HB	AfricaRice	125/125	119/119	174/174	132/132	195/195	204/204	196/196	210/210	157/157	185/189
42	GIGANTE X K85	NaCRRI	122/122	119/119	182/182	132/151	195/253	200/200	181/181	231/276	157/159	174/174
43	ART3-11L1P2-B-B-2	IRRI	125/125	119/119	174/174	184/184	195/195	204/204	201/201	210/259	163/163	188/191
44	WC-50 IR 77372-7	IRRI	122/122	119/119	170/170	126/126	217/217	210/210	168/168	228/231	157/157	174/177
45	K85 X WAC116	NaCRRI	122/122	121/121	182/182	146/146	217/217	210/210	165/165	231/285	157/157	174/174
46	WAB 2099-WAC1-TGR5-B	AfricaRice	122/122	121/121	182/182	null	null	210/210	168/168	210/231	157/157	155/157
47	IRAT325/WAB 450-B-136-HB	IRRI	125/125	119/119	174/182	132/132	195/195	204/204	165/176	210/231	155/157	174/189
48	WAB 2056-1-FKR4	AfricaRice	122/122	121/121	182/182	126/126	217/217	210/210	138/189	228/231	137/137	174/177
49	K5 X GIGANTE	NaCRRI	148/148	121/121	168/168	132/149	257/259	200/200	176/176	216/231	157/157	177/177
50	NERICA 4 X GIGANTE	NaCRRI	122/125	119/145	168/182	151/151	195/195	200/204	148/189	231/276	157/157	189/189
51	Jaribu	AfricaRice	112/122	121/121	182/182	160/160	217/217	210/210	148/189	210/260	157/157	177/177
52	BGM-117	IRRI	112/122	121/121	182/182	126/126	253/255	200/200	148/201	216/260	157/157	177/177
53	BGM IR 09L 223	IRRI	112/122	119/119	182/182	126/126	253/255	200/200	148/201	216/260	157/157	177/177
54	BGM-111	IRRI	112/122	121/121	182/182	164/164	249/249	210/210	148/189	231/257	157/157	177/177
55	NM7-27-1-B-P-77-6	NaCRRI	112/125	117/117	174/174	164/164	247/249	210/210	148/189	231/257	157/157	174/177
56	WAB 1436-20N-3-B-FKR2-WAC1	AfricaRice	112/122	121/121	182/182	164/164	247/249	210/210	148/189	231/257	157/157	174/177
57	NERICA 1 X WAC 117	NaCRRI	112/122	117/117	168/168	168/195	195/195	204/204	148/189	231/257	157/157	174/177
58	NERICA 6 X K5	NaCRRI	125/125	119/119	174/174	138/146	195/195	202/202	148/196	210/239	155/155	191/194
59	NERICA-L49	AfricaRice	112/122	121/147	182/182	146/146	217/217	210/210	168/168	231/257	159/159	159/174
60	IR 77372-3-B-6-2-BB	IRRI	122/125	119/119	174/174	132/132	195/195	204/204	148/201	210/210	157/157	185/189
61	IR 83383-B-B-141-AER	IRRI	112/122	121/121	182/182	166/166	247/249	210/210	189/189	231/257	157/157	180/182
62	CEHIRANG	AfricaRice	112/122	121/121	182/182	146/146	217/217	210/210	168/168	231/257	157/157	185/189
63	TXD 306 (NaCRRI)	NaCRRI	112/148	119/119	168/168	166/166	244/244	210/210	146/189	231/257	147/147	174/177
64	WAC 116	AfricaRice	122/125	119/119	174/174	132/132	195/195	204/204	196/196	210/210	157/157	185/189
65	NERICA-L49	AfricaRice	112/122	121/121	182/182	146/146	217/217	210/210	168/168	231/257	159/159	160/174
66	IR 77372-3-B-6-2-BB	IRRI	112/122	119/119	170/170	160/160	217/217	210/210	148/168	231/283	157/157	180/183
67	NERICA-L50	AfricaRice	112/122	121/121	182/182	146/146	217/217	210/210	165/165	231/285	157/157	174/174
68	AER-41	IRRI	112/122	119/119	170/170	146/146	249/251	210/210	148/201	216/257	157/157	174/177
69	BGM-100	IRRI	112/122	121/121	170/170	146/146	217/217	210/210	168/168	234/257	155/155	174/177
70	NERICA 1 X K85	NaCRRI	112/122	121/121	182/182	146/146	217/217	210/210	168/168	231/257	157/157	155/157
71	NERICA 1 X K5	NaCRRI	122/125	119/119	182/182	132/132	195/195	204/204	null	null	149/160	236/236
72	MET 113	AfricaRice	122/148	121/121	170/170	129/168	251/253	202/202	189/189	231/257	157/157	155/157
73	IR 84852-B-44-1-4 AER	IRRI	122/122	119/119	168/168	146/146	217/217	210/210	189/189	216/257	157/157	177/177
74	K 85	NaCRRI	122/122	119/119	182/182	153/153	257/259	200/200	181/181	228/285	157/157	174/174
75	NM7-8-2-B-P-2-1	NaCRRI	125/125	87/123	174/174	null	null	225/225	161/196	210/280	161/161	185/189
76	IR-77372-7-B-4-1-1-B	IRRI	122/122	119/121	182/182	146/168	217/257	200/210	165/181	228/231	157/157	174/180
77	WAB 2101-WAC3-1-TGR1-WAT B7	AfricaRice	122/122	121/121	182/182	146/146	217/217	210/210	165/165	231/285	157/157	174/174
78	BGM-5	IRRI	122/122	121/121	182/182	146/146	217/217	210/210	165/165	231/285	157/157	174/174
79	GIGANTE	Farmers	null	117/117	null	132/132	251/253	200/200	181/181	231/276	157/157	150/157
80	Supa	Farmers	122/122	121/121	170/170	168/168	251/253	210/210	176/176	237/263	147/147	183/185

Table 1. Contd.

81	K 5	NaCRRI	122/122	119/119	182/182	146/146	217/217	210/210	168/168	231/257	157/157	174/177
82	NERICA 1 X GIGANTE	NaCRRI	122/125	121/121	182/182	132/132	195/195	204/204	181/181	210/210	157/157	155/157
83	IR 79253-55-1-4-6AER	IRRI	122/125	123/123	174/174	146/166	217/217	210/210	148/189	231/257	157/157	177/177
84	NERICA 6 X NERICA 1	NaCRRI	122/122	119/119	182/182	190/192	195/195	200/200	148/196	210/239	147/155	185/189
85	IR-80353-63-B-38-1-2-B	IRRI	122/122	121/121	182/182	146/146	217/217	210/210	138/201	234/254	137/137	174/177
86	IR 09N 505	IRRI	122/122	119/121	182/182	146/146	217/217	210/210	189/189	231/257	157/157	174/177
87	NM7-9-2-B-P-2-1	NaCRRI	122/122	130/130	174/174	129/129	257/259	200/200	181/181	228/285	157/157	185/189
88	IR 83372-B-B-133-2	IRRI	122/122	121/121	182/182	146/146	257/259	210/210	168/168	231/257	165/167	180/183
89	IR 77372-3-B-6-2-BB	IRRI	125/125	119/119	174/174	184/184	195/195	204/204	148/201	210/259	168/168	191/191
90	FAROX 503-3-11-F43-2-1	IRRI	112/122	119/119	182/182	166/166	229/229	210/210	168/168	231/257	157/157	177/177
91	MET 103	AfricaRice	125/125	130/130	176/176	197/199	195/195	204/204	183/183	210/210	157/157	185/185
92	WAB 2151-TGR2-WAT B3	AfricaRice	122/122	121/121	182/182	146/146	217/217	210/210	168/168	231/257	159/159	157/174
93	IR-77381-2-1-7-1-1-B	IRRI	null	130/130	176/176	197/199	195/195	204/204	183/183	210/210	157/157	182/185
94	SK-19-38-2	AfricaRice	null	130/130	170/170	129/129	157/159	200/200	176/176	234/257	153/153	180/180
95	NM7-30-4-B-P-80-8	NaCRRI	125/125	119/119	174/174	132/132	195/195	204/204	148/196	210/210	157/157	185/189
96	WAB2066-6-FKR4-WAC1-TGR1-B-WAT-B1	AfricaRice	122/122	121/121	182/182	146/146	217/217	210/210	165/165	231/285	157/157	174/174
97	WAB 2076-WAC2-TGR1-B	AfricaRice	122/122	121/121	182/182	146/146	217/217	210/210	165/165	210/231	157/157	171/174
98	PVS-101	IRRI	122/122	121/121	182/182	146/146	217/217	210/210	165/165	231/285	157/157	174/174
99	BGM-73	IRRI	148/148	121/121	168/168	126/126	247/247	210/210	148/189	210/210	167/167	174/177

Chr = Chromosome.

Multiplexing and genotyping

The forward primer for each of the remaining polymorphic markers was labeled at the 5' end with one of the following fluorescent dyes: 6-FAM (Blue), VIC (Green), NED (Yellow), and PET (Red) (Life technologies, USA). To increase the throughput of standard SSR analysis, which yields genotypic information at only one locus per reaction, multiplex PCR was done to boost genotyping by amplifying three or four loci in the same reaction (Guichoux et al., 2011). Primers that have the same annealing temperature were given different dye colors since the alleles they amplify overlap (Masi et al., 2003). These multiplexed primers were used together in the same PCR cocktail to make one panel (Table 2).

Capillary electrophoresis and allele calling

The MicroAmp plates were fed into a 24 capillary genetic analyzer 3500 GeneScan (Life Technologies, USA). Four injections into each 96-well plate, lasted 45 min each. The fragments generated were analyzed using GeneMapper V4.1 software (Life Technologies) to score exact lengths of the alleles (Applied Biosystems Inc. USA). A single peak was detected at a particular SSR locus for a homozygote or a pair of peaks for a heterozygote. The internal lane size standard GeneScan-500 LIZ was used to automatically calculate the fragment sizes that range from 35 to 500 base pairs. The size standard peaks corresponding to 35 to 90 base pairs were excluded from the analysis because of their proximity to primer peaks (Life Technologies, 2014). To reduce genotyping errors and increase the precision of allele sizing, the auto-bining method of GeneMapper V4.1 was used to create bins which represented mean sizes of alleles (in base pairs) of a particular allele category. These adjusted bins were embedded into GeneMapper V4.1 and were used to correct allele sizing (allele calling) when data was reanalyzed (Life Technologies, 2014).

Data analysis

The population structure of the samples/genotypes was determined using a model-based program, STRUCTURE V2.3.4 (Pritchard et al., 2000). The maximum number of populations for the simulation was set at 10 (K=10), the length of Burnin period at 5,000 and the number of Markov chain Monte Carlo (MCMC) repetitions after analysis equal at 50,000. The Admixture model of the program was used and allele frequencies were correlated. The limit for assigning a sample to a particular population was set at 75% genomic ancestry. The genetic distance between the genotypes was calculated with DARwin V5 software, using simple matching method. A phylogenetic tree was built using the Unrooted Weighted Neighbor-Joining (UWNJ) algorithm. Statistical parameters were set using GenAlEx 6.5 software Peakall and Smouse, 2006), to define molecular diversity, such as heterozygosity, fixation index, Shannon's information index and the analysis of molecular variance (AMOVA).

RESULTS AND DISCUSSION

Allelic diversity of the rice genotypes

The SSR markers used were able to determine

Table 2. Three multiplex panels consisting of 10 rice SSR markers were used in this study for diversity analysis. SSR marker allele sizes, fluorescently labeled dye, repeat motifs, chromosome no and annealing temperature information are mentioned.

Panel	Marker	Annealing temp. (°C)	Chromosome no.	Allele size range (bp)	Dye color	Repeat motif
1	RM7	55	3	140-190	VIC	(GA)n
	RM249	55	5	100-160	NED	(GA)n
	RM125	62	7	100-150	6-FAM	(CTT)n
2	RM206	55	11	110-190	VIC	(GA)n
	RM273	55	4	160-220	NED	(TAC)n
	RM252	55	4	140-270	6-FAM	(TC)n
3	RM223	60	8	110-180	VIC	(CT)n
	RM333	60	10	120-190	NED	(TAT)n(CTT)n
	RM20A	60	12	200-280	6-FAM	(TAA)n
	RM154	60	2	110-190	PET	(CTT)n

The labeled SSR markers were synthesized by Life Technologies Inc., USA.

Table 3. Estimated genetic diversity parameters obtained at each locus across 99 genotypes.

Marker	No. of Alleles	He	Ho	I	F	Fst
RM125	7	0.554	0.216	0.950	0.616	0.120
RM249	9	0.481	0.075	0.862	0.845	0.192
RM7	6	0.600	0.013	1.055	0.980	0.094
RM206	24	0.707	0.144	1.624	0.815	0.111
RM252	16	0.659	0.212	1.378	0.701	0.141
RM273	7	0.466	0.035	0.854	0.928	0.167
RM154	21	0.809	0.412	1.928	0.474	0.074
RM20A	23	0.769	0.746	1.762	0.041	0.047
RM223	16	0.516	0.079	1.071	0.848	0.103
RM333	30	0.727	0.612	1.763	0.089	0.094
Mean	15.9	0.629	0.254	1.325	0.620	0.114
Std. Error	0.891	0.032	0.043	0.099	0.061	0.014

He=expected heterozygosity, Ho=observed heterozygosity, I=Shannon's information index, F=fixation index, Fst=fixation index statistic.

determine diversity in the rice genotypes (Table 3). All 10 markers were polymorphic, detecting a total of 159 alleles ranging from 6 to 30 alleles per locus with an average of 15.9±0.9 alleles/locus, clearly indicating that this set of 10 markers revealed a high level of genetic variability throughout the germplasm. The markers were picked from different linkage groups (chromosomes) of the rice genome and from the results, they amplified a varying number of alleles. The number of alleles observed in this research for markers RM125 (7) and RM7 (6) are comparable to those noted by earlier researchers on African rice (Drame et al., 2011). However, there were more alleles revealed in this study for markers RM333 (30 alleles), RM154 (21 alleles) and RM249 (19 alleles), indicating greater diversity in the materials used. This could be due to the use of breeding materials generated by intercrossing IRRI, AfricaRice and CIAT lines, thus increasing their level of diversity or due to materials having different origins. Other studies that considered different origins include those of Semon et al. (2005) who worked on 198 *Oryza glaberrima* accessions from 12 countries using 93 SSR markers, and reported an average of 9.4 alleles, Drame et al. (2011) studied 74 *O. glaberrima* samples from 9 countries used 30 SSR markers, and reported an average of 8.4 alleles. These differing results can probably be explained by differences in the sampling and number of markers used in the study. The most informative markers were RM 333, RM 20A and RM 206 since they were able to identify rare alleles and therefore give the highest numbers at 30, 23 and 24

alleles, respectively.

For all loci, the observed Heterozygosity (Ho) was lower (mean = 0.254 ± 0.043) than the expected Heterozygosity (He) (mean = 0.629 ± 0.032), suggesting a clear shift from the Hardy-Weinberg equilibrium. This shift can only be attributed to forces akin to inbreeding within groups (Masudaab et al., 2009) or lack of distinctly isolated populations of the available rice germplasm in Uganda. The Fst values were generally low, ranging from 0.047 to 0.192, indicating that low levels of genetic differentiation were present in the populations sampled from IRRI, AfricaRice and NaCRRI, as reported by Ogumbayo et al. (2005) regarding genotypes from AfricaRice.

Diversity groups of Uganda rice genotypes

Grouping of the rice populations was determined using ancestry-based grouping and a phylogenetic tree identifying the different groups. Using a cluster analysis of the allelic data (Figure 1), five genetic groups were identified in the rice germplasm used in the study. The classification of the genotypes was in agreement with their parentage, which comprised of *Aus*, *Indica*, tropical *japonica*, temperate *Japonica* and *Basmati* groups of rice. A similar population structure was also documented by Garris and Ni (Garris et al., 2005; Ni et al., 2002). Garris et al. (2005) reported 5 populations that corresponded to *indica*, *aus*, *aromatic*, *temperate japonica* and *tropical japonica* using samples from Asia, the Americas and Africa. The statistical program used in this study (STRUCTURE V2.3.4) tends to give more populations that are biologically relevant. Therefore, to ascertain the identified groups, the method described by Evanno et al. (2005) was used.

This method tests the number of populations that are statistically significant in the samples when patterns of dispersal among them are not homogeneous as in the case of allelic variability (Evanno et al., 2005). By using the log probability for the rate of change of the data (ΔK) between values of successive K (number of populations), STRUCTURE gave the accurate number of populations (K) at K=5. The results, presented in Figure 2, revealed the structure of the rice genotypes showing five populations consisting of admixed genotypes. This admixture in populations is probably because rice breeding centers share germplasm freely and make crosses with whichever cultivars show desirable traits, resulting in common alleles in all populations (Ni et al., 2002). This gene flow (gene migration) causes a marked change in allelic frequency (Beringer, 2003), so that alleles of various individuals end up being present in all populations. There was therefore no “island” population of rice genotypes observed in this study, indicating that the genetic base was small, as suggested by Cuevas-Pe´rez et al. (1992).

The genotypes grouped according to differences in their alleles, revealed one extra group compared to Drame et al. (2011) who reported four distinct genetic groups of African rice samples using samples of wild rice (*O. glaberrima*, *Oryza longistaminata* and *Oryza barthi*) and 6 cultivated genotypes. However, the present study used cultivated rice, some of which were NERICA varieties that are crosses between wild and cultivated rice, and therefore were expected to have broader genetic diversity than shown in the earlier studies by Drame et al. (2011). The groups observed in this study were not clearly separated from each other, due to admixtures, which suggested that most of them were crosses with each other. Genotypes 11 (IR 83372-B-B-133-2), 3 (BGM) and 57 (NERICA 1/WAC 117) clustered alone in group 1 (Figure 1) suggesting a unique group of alleles that was not present in other groups, and thus indicating that this group could be useful as parents to broaden the diversity of rice breeding materials (Cuevas-Pe´rez et al., 1992).

Assigning genotypes to their centers of origin

The AMOVA results are presented in Table 4, revealing most of the variation among individual genotypes (56%) and within individuals (41%). Only 3% of the variation was associated with the origins of the genotypes, suggesting that the diversity available among and within the rice genotypes was sufficient to be used for improving rice productivity in Uganda. The four origins of the 99 rice genotypes used in this study were; NaCRRI (61), IRRI (24), AfricaRice (12) and Ugandan farmers (2) and analysis using GenAlEx software was expected to assign the genotypes to their respective centers of origin. The results (Table 5) indicated that the different centers of origin of the genotypes, as their fixed population in the analysis, were assigned 53% while 47% of them were assigned to other populations (Other) at $P < 0.1$. The markers used were able to differentiate half of the genotypes used by their centers of origin. The other 47% were assigned different origins probably because they resulted from crosses between genotypes that could have been introduced from other centers (Guimaraes, 2009). The origin of neither of the two varieties obtained from farmers in Uganda (Supa and Sindano) was assigned to farmers, and remains unclear (Kijima et al., 2012) though they are believed to have originated from Tanzania (Kijima et al., 2012) where Sindano is a landrace (FAO, 1987).

Conclusion

The use of molecular markers to determine genetic diversity in the germplasm is demonstrated as a feasible approach in Uganda. The SSR markers RM 333, RM 20A and RM 206 were the most informative in this research.

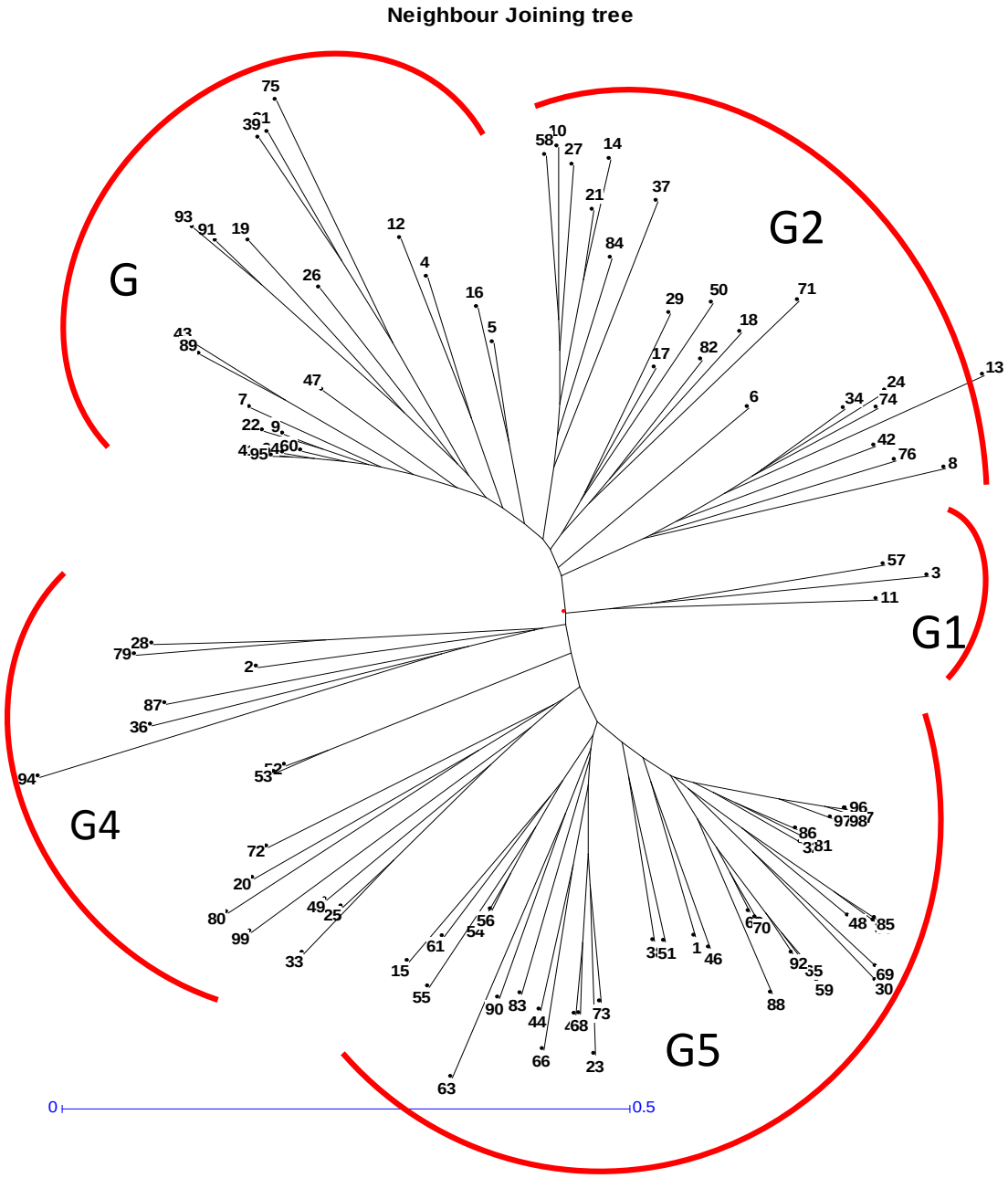

Figure 1. UWNJ tree cluster analysis using DARwin V5 software.

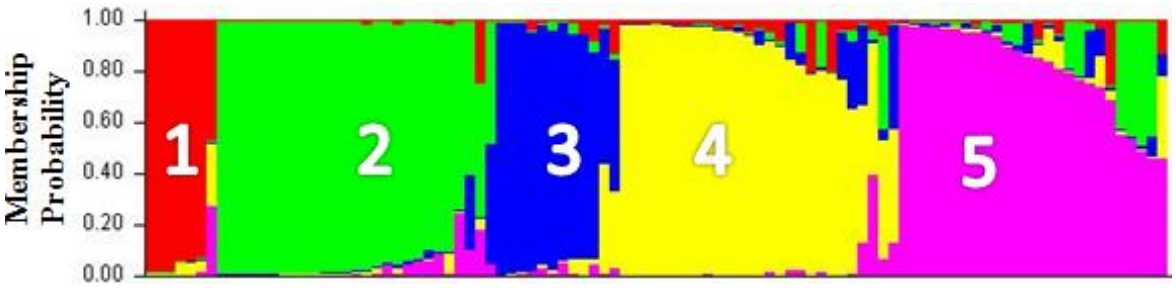

Figure 2. Population Structure of 99 rice genotypes at k=5.

Table 4. Summary of AMOVA in the genotypes.

Source	df	SS	MS	Est. Var.	%Var.
Among Origins	3	28.633	9.544	0.102	3%
Among Individuals	95	555.716	5.850	2.147	56%
Within Individuals	99	154.000	1.556	1.556	41%
Total	197	738.348		3.805	100%

A probability value of 0.001 was used based on permutation across the full data set.

Table 5. Assignment of population to origin or "other" categories.

Population	Genotypes in Population	Origin Population assignment	Other Population assignment	Probability level
NaCRRI	61	41	20	
IRRI	24	8	16	
AfricaRice	12	3	9	
Uganda-Farmers	2	0	2	
Total	**99**	**52**	**47**	
Percent	100%	53%	47%	99%

The rice germplasm from IRRI, AfricaRice and NaCRRI was assigned 5 groups.

Conflict of interests

The authors declare that there are no conflicts of interests

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Abbreviations: IRRI, International Rice Research Institute; **SSR,** simple sequence repeat; **NaCRRI,** national crops resources research institute; **RAPD,** random amplified polymorphic DNA; **AFLP,** amplified fragment length polymorphisms; **RFLP,** restriction fragment length polymorphisms; **SNP,** single nucleotide polymorphisms; **BAC,** bacterial artificial chromosome; **PAC,** P1-derived artificial chromosome; **PCR,** polymerase chain reaction; **Ho,** heterozygosity; **He,** heterozygosity.

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