Vol. 10(10), pp. 262-272, October 2018
DOI: 10.5897/JPBCS2018.0760
Article Number: FD3FC1358609
ISSN: 2006-9758

http://www.academicjournals.org/JPBCS

ACADEMIC JOURNALS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Assessment of salt tolerance and variability within some rice germplasm using microsatelittes

Anyomi W. E.*, Ashalley R., Amoah N.K.A, Blay E.T. and Ofori K.

Department of Crop Science, College of Basic and Applied Sciences, University of Ghana, P. O. Box Lg 44, Legon, Accra, Ghana.

Received 12 July, 2018; Accepted 21 August, 2018

Soil salinity is a major abiotic stress that affects rice production. It can reduce yield drastically and result in total crop failure. The objectives of this study are to determine the genetic diversity within thirty-six rice accessions and to identify genotypes that are tolerant to salinity. Thirty-six rice accessions including three check varieties were sown in experimental pots and their leaves harvested for DNA extraction. Screening was done with 31 simple sequence repeats (SSRs) primers, of which 14 were markers for salt tolerance, 2 primers did not produce any results. 28 out of the 31 primers were polymorphic. The polymerase chain reaction (PCR) products were run and visualized on a 3% agarose gel matrix stained with ethidium bromide. Amplified bands were scored and analyzed with PowerMarker v3.25 and DARwin v5 software. The genetic diversity among the accessions assembled was high (He=0.6, I=0.516, PIC=0.471). Saltol primers RM10711 and RM10793 were the only primers able to completely discriminate tolerant genotypes from susceptible ones, hence they can be used in selections involving the genotypes. Accessions SR1, IR72, Sebota 337-1, Perfume (Short) type, Anyofula, Local Red, GR18Red, GH1580, GH1528, GH1575, NericaL23, NericaL24 and NericaL27 performed well under salinity stress in this study and were identified to be superior among the accessions used. These accessions should be incorporated into major breeding programs to improve the salt tolerance of existing commercial lines or for the production of new commercial lines.

Key words: Rice, abiotic stress, agarose gel, genetic diversity, polymerase chain reaction (PCR), simple sequence repeats (SSRs) markers, salinity.

INTRODUCTION

Rice (*Oryza sativa* L. and *O. glaberima* Steudl) is an important cereal of the Poaceae family grown worldwide. Two species of importance in the genus are namely, *O. sativa* the universally cultivated Asian rice, and *Oryza glaberrima,* the West African cultivated rice. African rice is now only rarely grown in pure stands. It is currently grown in mixture with the Asian rice in various proportions. The extent of even this form of mixed cultivation is diminishing as it is being replaced with 'pure' Asian rice (Nayar, 2010). Rice is the fastest growing food source in Africa (Nwanze et al., 2006). Rice has become a major staple crop in recent decades with a per capita consumption of 25 kg/annum in Ghana, but most of the consumption is met by imports (MOFA, 2010). In 2009,

*Corresponding author. E-mail: lordedems@gmail.com.

Ghana imported over 350,000 tons of milled rice worth 600 million US dollars (Duffuor, 2009).

Salinity of arable land is one of the most important factors retarding rice growth and development at both vegetative and reproductive stages (Zeng and Shannon, 2000; Zeng et al., 2003). Salinity is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, rising to 50% by the year 2050 (Wang et al., 2003). Salinity reduces plants ability to take up water and results in growth reduction. Excess salts in plants can reach toxic levels, which causes premature leaf senescence and ultimately photosynthesis reduction (Munns, 2002). Specific effects of salt stress on plant metabolism, especially on leaf senescence, have been related to the accumulation of toxic Na+ and Cl- ions and to K+ and Ca^{2+} depletion (Al-Karaki, 2000). Salinity associated with excess NaCl adversely affects the growth and yield of plants by depressing the uptake of water and minerals and normal metabolism (Akhtar et al., 2001; Akram et al., 2001).

Salinity has been found to negatively impact a number of yield components of rice including stand establishment; panicles, tillers and spikelets per plant; floret sterility; individual grain size; and even delayed heading. Maas and Grattan (1999) and Hanson et al. (1999) indicated that rice yields decrease by 12% for every unit (dsm^{-1}) increase in EC (average root-zone EC of saturated soil extract) above 3.0 dsm^{-1}. Plant breeding harnesses inherent variability within plants for economic gains. It involves redistributions of genes in a population. In so doing, genes of interest are propagated whiles others are eliminated from the population.

In breeding for salinity tolerant varieties there is a need to screen and select tolerant varieties. The success of salt tolerance breeding programs employing traditional screening and selection has some limitations. Conventional methods of plant selection for salt tolerance are difficult because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997). Genetic improvement of salt tolerance in rice using marker assisted selection (MAS) is most feasible and promising strategy (Munns, 2002). SSR are ideal genetic markers (Farooq and Azam, 2002) and have a repeat-unit length of 1-6 base pair units arranged in repeats of mono-, di-, tri-, tetra and penta-nucleotides (A,T, AT, GA, AGG, AAAG) with different lengths of repeat motifs. According to Mason (2015) and Joshi et al. (2011), SSRs are highly informative, codominant, multi-allele genetic markers that are experimentally reproducible and transferable among related species. The variation in the number of tandemly repeated units results in highly polymorphic banding pattern (Farooq and Azam, 2002) which are detected by PCR, using locus specific flanking region primers where they are known. Microsatellite markers have been used to identify the variation among rice cultivars (Yang et al., 1994; Akagi et al., 1997; Garland et al., 1999). Similarly, Thanh et al. (1999) showed the genetic variation identified by microsatellite markers to be useful in evaluating upland rice accessions. Genetic diversity between parental genotypes is usually estimated by measurements of physiological and morphological differences of quantitative and economically important traits. Diversity ensures a large genepool from which traits can be mined for economic gains. Without diversity, a species finds it difficult to adapt to the ever changing environmental and biotic stresses. With wide range of crops, a breeder is able to screen and choose materials for various purposes. The objective of this study was therefore to determine diversity among thirty-six accessions of rice and to identify salt tolerant genotypes using SSR markers.

MATERIALS AND METHODS

Experimental site

The research was carried out at the Biotechnology Center of the Department of Crop Science, University of Ghana, Legon.

Experimental materials

Plant materials were obtained from the Plant Genetic Resources Research (PGRRI) Institute and Savanna Agricultural Research Institute (SARI), both for the Council for Scientific and Industrial Research (CSIR), Ghana. NERICA rice genotypes were also obtained from Africa Rice Center, Sahel Station- Senegal, and from farmers' fields in Ghana. Table 1 shows the list of accessions and their sources. FL478 an International salt-tolerant accession and IR29, an international sensitive genotype obtained from Africa Rice Center were used as checks for salinity. Accession 'CG14' from Africa Rice Center was included as check for O. *glaberrima*, in the accessions from African countries. A total of 36 germplasm was investigated (Table 1).

Sowing of the rice accessions

The seeds of the 36 accessions of rice were nursed in a nursery pot at the green house in the department of crop science, Legon. The leaves of the accessions were collected for DNA extraction.

DNA extraction for molecular studies

DNA was extracted using E.Z.N.A. ™ SP Plant DNA Mini Kit. Approximately 0.03 g of leaf samples was frozen in liquid nitrogen and ground in a microfuge tube. 400 µl of buffer SP1 was immediately added followed by 5 µl of RNase. The samples were incubated at 65°C for 10 min. 140 µl of buffer SP2 was added to each sample and mixed vigorously by vortexing. This was followed by incubation on ice for 5 min and centrifugation at 14000 rpm for 10 min. The supernatant that resulted was carefully aspirated into an Omega® Homogenizer Column placed in 2 ml collection tube and centrifuged at 14000 rpm for 2 min. 500 µl of the clear lysate that resulted was transferred into a 1.5 ml tube. Binding conditions of the sample were then adjusted by pipetting 750 µl of buffer sp3/ ethanol mixture directly onto the clear lysate.

650 µl of the resulting mixture was transferred into a Hiband® DNA Mini Column placed in a 2 mL collection tube and centrifuged

Table 1. The germplasm studied and their sources.

Accession number	Name	Source
1	GH 1593	CSIR-PGRI, Ghana
2	GH 1575	CSIR-PGRI, Ghana
3	GH1585	CSIR-PGRI, Ghana
4	GH1598	CSIR-PGRI, Ghana
5	GH1571	CSIR-PGRI, Ghana
6	GH 1533	CSIR-PGRI, Ghana
7	GH 1528	CSIR-PGRI, Ghana
8	GH1545	CSIR-PGRI, Ghana
9	GH 1580	CSIR-PGRI, Ghana
10	GH 1599	CSIR-PGRI, Ghana
11	SR-1	ARC- Senegal
12	IR-29	ARC-Senegal (Susceptible check)
13	CG14	ARC- Senegal (*O. glaberrima*)
14	FL478	ARC- Senegal (Tolerant check)
15	Nerica L23	ARC- Senegal
16	Nerica L9	ARC- Senegal
17	Nerica L24	ARC- Senegal
18	Nerica L27	ARC- Senegal
19	Sebota 33	Cameroun
20	Sebota 337-1	Cameroun
21	Perfume (Short type)	Thailand
22	Sebota 41	Cameroun
23	Local Red	Farmer collection, E/R
24	Anyofula	CSIR-PGRI, Ghana
25	Good and New (JP)	Japan
26	IR 72 (Ph)	IRRI, Philippines
27	GH 1837	CSIR-PGRI, Ghana
28	Matigey	CSIR-PGRI, Ghana
29	Basmati 122	IRRI, Philippines
30	GR 18 Red	CSIR-SARI, Ghana
31	Local Basmati-2	IRRI, Philippines
32	Koshihikari	Japan
33	Viwornor	CSIR-PGRI, Ghana
34	Sebota 281-2	Cameroun
35	Sebota 68	Cameroun
36	Abidjan	Local farmer

for 1 min at 14000 rpm. Later, the flow through was discarded. This was repeated for the remaining mixture. The columns were then placed into a new 2 ml collection tube and 650 µl of SPW Wash Buffer diluted with ethanol was added. This was centrifuged at 14000 rpm for 1 min and the flow through was discarded. This step was repeated with the sample volume of SPW wash buffer. The empty column was centrifuged at 14000 rpm for 2 min. The Hiband® Mini column was then transferred into a sterile 1.5 ml tube and 100 µl of pre-warmed (65°C) elution buffer was added. This was then centrifuged at 14000 rpm for 1 min to elute the DNA.

Polymerase chain reaction (PCR) amplification

Thirty-One SSR primers (Table 2), 15 of which were markers for salt tolerance were selected for the PCR. 15 µl PCR reaction was composed of 1X Taq buffer, 2 mM $MgCl_2$, 1U Taq DNA polymerase, 0.2 mM of each dNTP's and 0.4 µM SSR primer pair. Deionized water was used to make up the volume to the final PCR reaction volume. The thermal cycling conditions were as follows; 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 1 min for annealing temperature (55-67°C) depending on the primer used and 72°C for 2 min, and a final extension at 72°C for 5 min. Primers were selected from previous works of Deepti et al. (2013), Huyen et al. (2012) and Thompson et al. (2010).

Gel electrophoresis of amplified products

For a 120 ml electrophoresis casting tray, 3.6 g of agarose was weighed into 120 ml of TAE buffer. The initial weight was noted. This was then melted on a hot plate after which distilled water was used to make up the weight difference. The melted gel was then cooled under running water after which 8 µl of ethidium bromide

Table 2. SSR primers used in PCR, their annealing temperatures and flanking sequences.

Primer	Ann. temp (OC)	Forward sequence (5'- 3')	Reverse SEQ. (5'-3')
RM20	55	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
RM307	55	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
RM5	55	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
RM552	55	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC
RM19	55	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
RM454	55	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
RM11	55	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG
RM518	55	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
RM334	55	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG
RM237	55	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
RM259	55	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
RM474	55	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG
RM178	67	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC
RM489	55	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG
RM312	55	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC
RM253	55	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
RM336	55	CTTACAGAGAAACGGCATCG	GCTGGTTTGTTTCAGGTTCG
RM10655	55	AGTACCGTTGAATCCGATATGC	TGGTTGAGGTGCTGAATTGG
RM10696	60	CCTTCGACTCCATGAAACAAACG	TCTCTTTGCCCTAACCCTATGTCC
RM10711	55	GCTTCGATCGATGAGAAAGTAGAGG	GAATCTCCCATCCTTCCCTTCC
RM10713	60	ATGAACCCGGCGAACTGAAAGG	CTGGCTCCCTCAAGGTGATTGC
RM10748	60	CATCGGTGACCACCTTCTCC	CCTGTCATCTATCTCCCTCAAGC
RM10722	60	GCACACCATGCAAATCAATGC	CAGAAACCTCATCTCCACCTTCC
RM10793	60	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC
RM10800	60	CGTACGCCCTCACATCACCTTTCC	CTCTCCGGGAGCTCACTTGTCG
RM10825	60	GGACACAAGTCCATGATCCTATCC	GTTTCCTTTCCATCCTTGTTGC
RM10843	60	CACCTCTTCTGCCTCCTATCATGC	GTTTCTTCGCGAAATCGTGTGG
RM10852	60	GAATTTCTAGGCCATGAGAGC	AACGGAGGGAGTATATGTTAGCC
RM10864	60	GAGGTGAGTGAGACTTGACAGTGC	GCTCATCATCCAACCACAGTCC
RM10890	60	GCTTCGGCTCTTCATTCACTGG	GCGATTATAGGAGCGCTATGTGG
RM10927	60	TGGATCCCACTAATCCAAATGC	GAAAGACTCCTTCCAATGTTAGGC

was added and swirled gently to mix. The agarose gel was then poured into the casting tray and combs set in place. This was allowed to solidify for 40 min. The casting tray together with the solidified agarose was then transferred into the electrophoretic tank and submerged with TAE buffer and the combs gently removed. 10 µl of PCR amplicons was mixed with 3 µl of 6X loading dye and carefully loaded into wells created by removing the combs. The leads of the electrophoretic tank were connected to electrophorese amplicons at 100V for 1 h after which the gel was viewed with the aid of a UV transilluminator.

Allele scoring and data analysis

The polymorphic bands were scored for each of the microsatellite primer pairs in each genotype based on presence 1 or absence 0 for bands to generate a matrix of 1 and 0. The size (in nucleotide base pair) of the amplified band for each SSR marker was determined based on its migration with comparison to a known molecular weight marker (1Kb DNA Ladder). Allele numbers, gene diversity, heterozygosity and polymorphic information content (PIC) were calculated with Power Marker v3.25 software (Liu and Muse 2005). Between samples, genetic distances were assessed through simple matching index as implemented in DARwin v5 software (Perrier et al., 2003). A dendogram was constructed based on the unweighted pair-group method with arithmetic averages (UPGMA) using the neighbor-joining (NJ) method as implemented in the same software. Polymorphic information content (PIC) values were calculated with the following formula (Anderson et al., 1993):

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

Where, n is the number of marker alleles for marker *i* and P*ij* is the frequency of the *j*th allele for marker *i.*

RESULTS

Microsatellite variations of the rice accessions

Salt tolerance SSRs

Twenty saltol SSR markers were screened; 16 produced

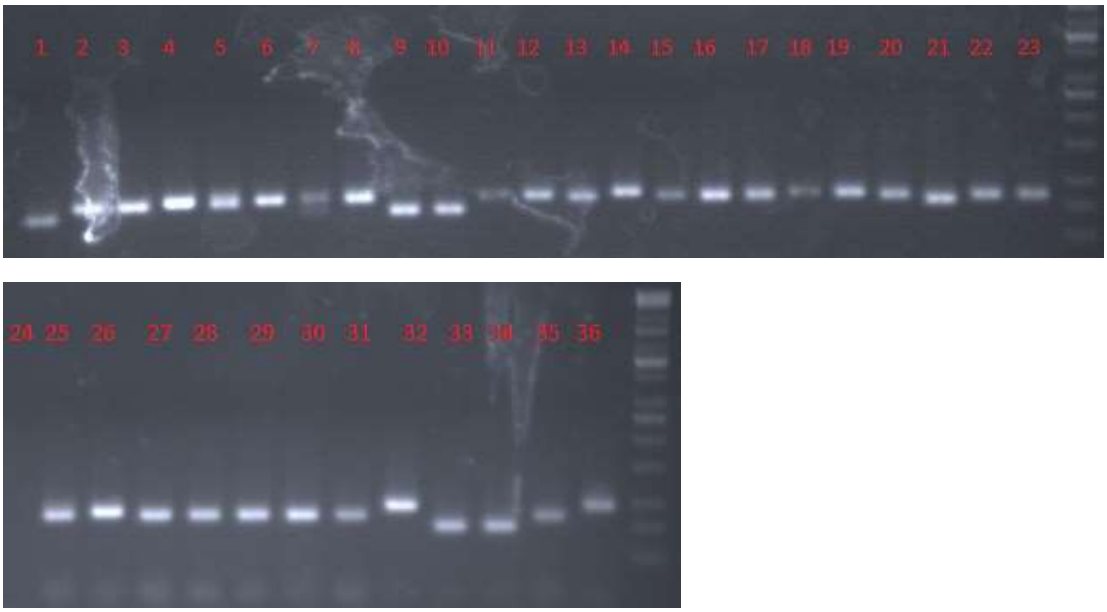

Plate 1. A gel image of the banding pattern of the genotypes with Primer RM10711. 1= Local Red, 2= Sebota 41, 3= Perfume (Short type), 4= Sebota 337-1, 5= Sebota 33, 6= Nerica L27, 7= Nerica L24, 8= Nerica L9, 9= Nerica L23, 10= FL478, 11= CG14, 12= IR-29, 13= SR-1, 14= GH1599, 15= GH1580, 16= GH1545, 17= GH1528, 18=GH1533, 19= GH1571, 20= GH1598, 21= GH1585, 22= GH1575, 23= GH1593, 24= Abidjan, 25= Sebota 68, 26= Sebota 281-2, 27= Viwornor, 28= Koshihikari, 29= Local Basmati-2, 30= GR 18 Red, 31= Basmati 122, 32= Matigey, 33= GH 1837, 34= IR 72 (Ph), 35= Good and New (JP), 36= Anyofula.

polymorphic bands. Primers RM10711 and RM10793 showed bands that differentiated the tolerant and susceptible checks. Primer RM10711 (Plate 1) was able to separate ten Ghanaian, three Nericas, two Basmati and three Sebota entries as susceptible. It also distinguished Koshihikari as susceptible. The primer recognized three entries as tolerant. Accessions Perfume (Short type), Sebota 41, Good and New (JP), GH 1528, Sebota 337-1, local Red, GH1545, GH 1580, SR-1 and Gh1585 did not share any bands with the two checks.

Primer RM10793 showed that accessions GH1599, SR-1, GH1571, GH1533, Nerica L9, Nerica L27, Perfume (Short type), GH1837, Matigey, Basmati 122, Sebota 281-2, Sebota 68 shared a common band with IR29 the susceptible check. GH 1575, GH1585, GH1598, GH 1528, GH1545, GH 1580, CG14, Nerica L23, Nerica L24, Sebota 33, Sebota 41, Local Red, Good and New (JP), IR 72 (Ph), Local Basmati-2, Koshihikari, Viwornor all had similar bands to FL478, the salt tolerant check. GH1593, Sebota 337-1, Anyofula, and GR 18 Red did not share bands with the two checks in relation to primer RM10793 (Plate 2).

Diversity among accessions

Out of the 31 SSR primers used, 28 produced polymorphic bands representing 84.8%. Fourteen out of the 28 SSR primers were located at the saltol loci of the rice genome, the remaining 14 spread throughout the entire genome. A total of 116 alleles with an average of 4.14 alleles per locus were generated by the 28 primers (Table 3).

The highest allele frequency was 100% produced by primer RM454 and the lowest allele frequency was 30% produced by primers RM10748, RM10864 and RM20. The overall average allele frequency was 60%. The Polymorphic Information Content, PIC, of the primers among the 36 rice genotypes was observed in the range of 0.053 to 0.785, with an average of 0.471. The genetic diversity within the population was 51.6% but RM20 had the highest diversity of 84.6% and RM454 the lowest of 5.4%. Primer RM20 had the highest PIC of 0.829 followed by RM10864 and RM10793 respectively, with RM454 having the least PIC of 0.053. Primer RM20 had the highest diversity discrimination of 84.6% followed by RM10864 with 78.5% and RM10793 with 75% respectively; while RM454 had the lowest diversity of 5.4%.

Genetic divergence of rice population as revealed by the dendrogram

A dendrogram was constructed based on the unweighted pair-group method with arithmetic averages (UPGMA) using the neighbor-joining (NJ) method. The dendrogram constructed grouped the accessions into clusters,

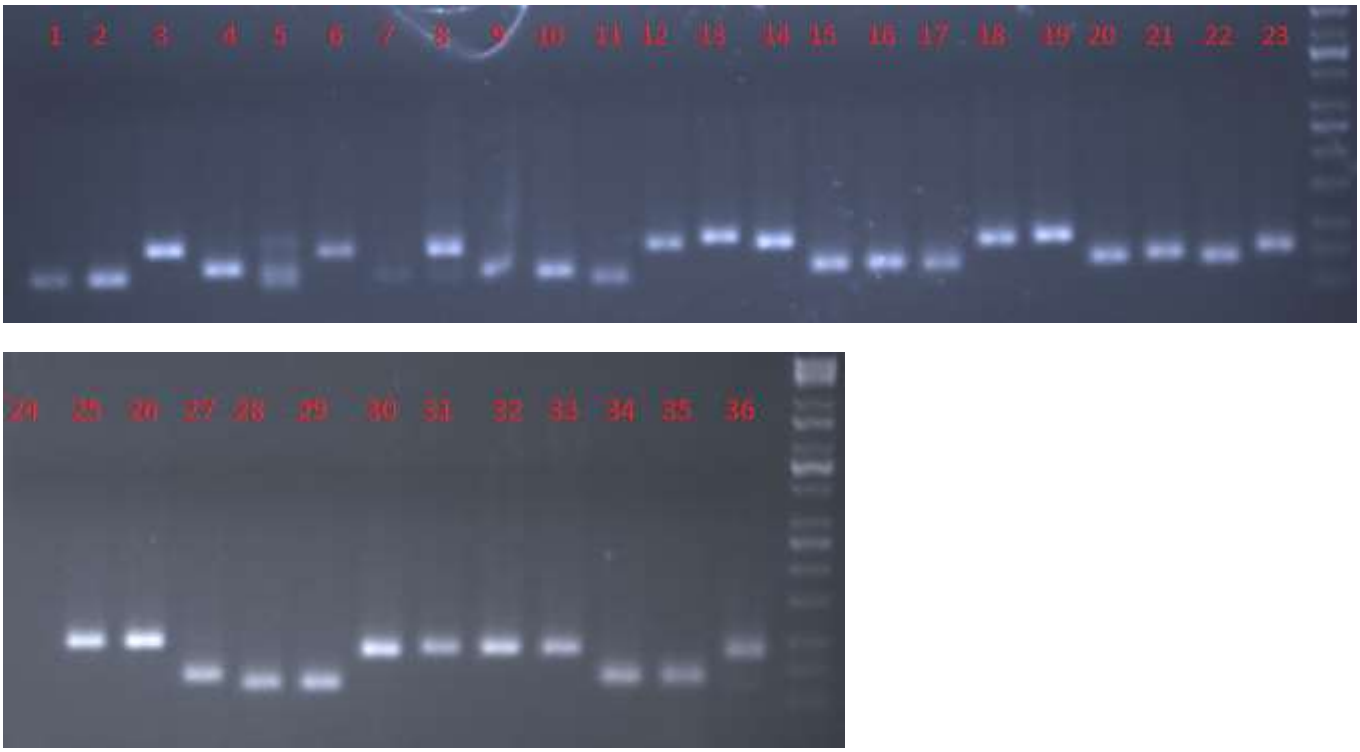

Plate 2. A gel image of the banding pattern of the genotypes with primer RM10793. 1= Local Red, 2= Sebota 41, 3= Perfume (Short type), 4= Sebota 337-1, 5= Sebota 33, 6= Nerica L27, 7= Nerica L24, 8= Nerica L9, 9= Nerica L23, 10= FL478, 11= CG14, 12= IR-29, 13= SR-1, 14= GH1599, 15= GH1580, 16= GH1545, 17= GH1528, 18=GH1533, 19= GH1571, 20= GH1598, 21= GH1585, 22= GH1575, 23= GH1593, 24= Abidjan, 25= Sebota 68, 26= Sebota 281-2, 27= Viwornor, 28= Koshihikari, 29= Local Basmati-2, 30= GR 18 Red, 31= Basmati 122, 32= Matigey, 33= GH 1837, 34= IR 72 (Ph), 35= Good and New (JP), 36= Anyofula.

indicating the diversity that existed between the accessions. The accessions were separated into three major clusters (Figure 1). Major Cluster 1 comprised Nine Ghanaian entries, all four Nerica's, one Sebota entry, one Thailand entry, one Japanese entry and the two checks. Major cluster 2 comprised eight Ghanaian entries including a collection from a farmer's field, four Sebota entries, the two Basmati entries from the Philippines, one Japanese entry and two entries from ARC Senegal. Major cluster 3 had only IR72 (Ph) from the Philippines. Major cluster 1 had two sub clusters. Sub cluster I and II, sub cluster I had two sub clusters, the first contained three Nerica entries and five Ghanaian entries together with the checks, the second cluster consisted of four Ghanaian entries only. All Nericas were separated from each other in this sub group. Sub cluster II had only two entries, Nerica L24 and Local Red

Major cluster 2 had two sub clusters III and IV. Sub cluster III had two lower clusters; the first comprised six Ghanaian entries, one Japanese and Philippine entry. The second contained only one entry from Sebota. Sub cluster IV had two sub groups; the first consisted of three Sebota entries, two Ghanaian entries and one Philippine entry. The second group had one entry from ARC Senegal.

DISCUSSION

Genetic diversity and identification of most informative markers

Out of the 31 SSR primers used, 28 produced polymorphic bands representing 90.03%. Fourteen out of the 28 SSR primers were located at the saltol loci of the rice genome, the remaining 14 were spread throughout the entire genome. A total of 116 alleles with an average of 4.14 alleles per locus were generated by the 28 primers. The average allele frequency was 0.6. The PIC ranged from 0.053 to 0.829, with an average of 0.471. The genetic diversity within the population was 51.6%. The highest diversity was 84.6% and the lowest was 5.4%. Similar results were recorded by Lang et al. (2008) where 95% of SSR markers for genetic diversity were reported to be polymorphic in IR64 variety. The results are also in line with Deepti et al. (2013), who reported a higher average PIC of 0.67 for 26 SSR markers within a range of 0.50 to 0.89. The number of alleles obtained per locus was 7.1, which was higher than the values obtained in this work. Mahalingam et al. (2013), on the other hand, reported an average PIC value of 0.44 lower than what was obtained in this study. Their highest PIC value

Table 3. SSR primers used with their parameters for diversity.

Marker	Major allele frequency	No of alleles	Gene Diversity	PIC
RM336	0.4	5	0.690	0.639
RM10655	0.4	5	0.708	0.660
RM10696	0.7	5	0.511	0.470
RM10711	0.4	5	0.719	0.668
RM10713	0.8	4	0.336	0.317
RM10722	0.4	6	0.728	0.689
RM10748	0.3	4	0.742	0.694
RM10793	0.4	7	0.750	0.711
RM10800	0.5	4	0.642	0.583
RM10825	0.5	4	0.640	0.592
RM10843	0.7	4	0.444	0.409
RM10852	0.5	4	0.625	0.568
RM10864	0.3	7	0.785	0.755
RM10890	0.7	2	0.444	0.346
RM10927	0.7	2	0.424	0.334
RM253	0.8	3	0.323	0.285
RM518	0.9	2	0.153	0.141
RM312	0.9	2	0.105	0.099
RM489	0.8	3	0.403	0.363
RM474	0.5	5	0.660	0.604
RM259	0.8	4	0.412	0.383
RM11	0.6	3	0.537	0.441
RM454	1.0	2	0.054	0.053
RM19	0.7	3	0.415	0.349
RM5	0.6	2	0.486	0.368
RM20	0.3	9	0.846	0.829
RM307	0.8	5	0.298	0.287
RM522	0.6	5	0.576	0.536
Mean	**0.6**	**4**	**0.516**	**0.471**

reported was greater than 0.60 and the lowest was 0.035; both lower than what was obtained in this experiment. The present estimate of PIC was also larger than that reported by Hashimoto et al. (2004) in a Japanese rice population comprising 171 cultivars used in brewing of Japanese rice wine; it had a diversity of 0.33.

Singh et al. (2011), in their genetic diversity study of rice genotypes using 30 SSR markers, noted fewer alleles (83) with a lower average of 2.76 alleles per marker; but they had a high PIC value varying from 0.54 to 0.96. Studies by Chakravarthi and Naravaneni (2006) revealed that primer RM20 on chromosome 12 had seven alleles. In the present study, primer RM20 had 9 alleles, indicating that it is very polymorphic and suitable for diversity studies. El-Malky et al. (2007) used 14 microsatellites to generate a total of 122 alleles with an average PIC of 0.782 and a range of 0.438 to 0.891. All their diversity parameters were higher than those obtained in this work. Islam et al. (2012) detected a total of 168 alleles; the number of alleles per locus ranged from 2 to 6 which was lower than what was obtained in this work (2 to 9); but they had an average of 4.2 alleles per locus slightly higher than the value obtained in this study.

Polymorphic information content (PIC) value varied from 0.21 to 0.76 with an average of 0.57 higher than that of this study, 0.471. Lapitan et al. (2007) reported higher parameters than those obtained in this study. They had a total of 176 alleles. Their number of alleles per marker was high ranging from 6 to 22, with an average of 14.6 alleles per locus. Their primers were thus very useful in distinguishing the germplasm used. Roychowdhury et al. (2013) also detected a total of 122 alleles which was higher than that obtained in this study but the primer used had a lower allele range of 2 to 5 alleles compared to this study (2 to 9). They also reported a lower average of 3.21 alleles per locus but the PIC value was 0.524 which was higher than the results from this study.

Emon et al. (2015) reported a total of 209 alleles among 5 rice genotypes using 160 SSR markers. They had a

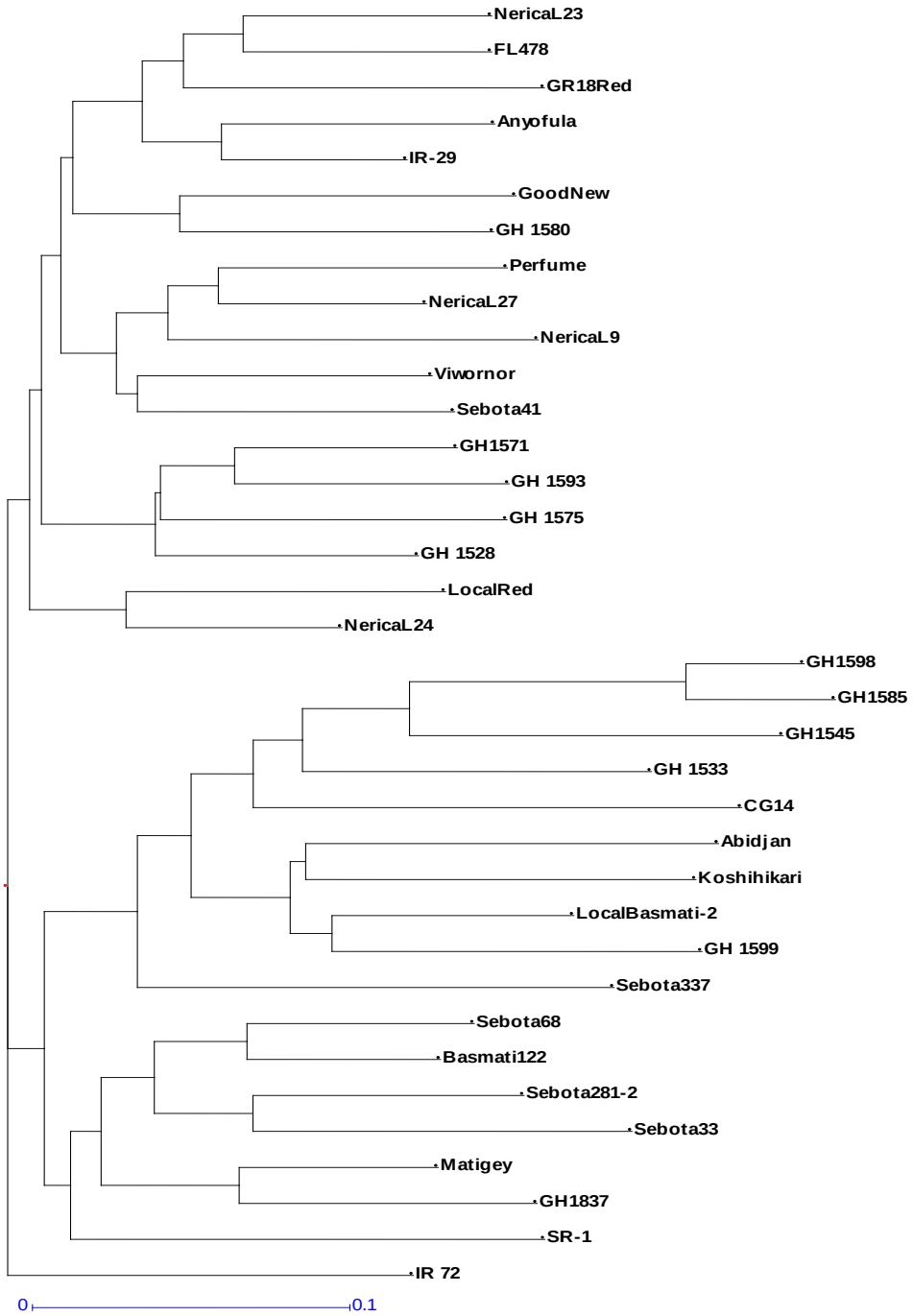

Figure 1. Dendrogram of the clustering of the genotypes with the SSRs markers.

lower PIC of 0.32 and also a lower diversity of 0.37. Ram et al. (2007) reported number of alleles per locus varied from 3 to 8, with average number of alleles per locus at 4.86. This indicates almost the same magnitude of diversity with reference to the markers used in this investigation. Behera et al. (2012) observed a total of 169 alleles, of which 166 were polymorphic from a set of 36 microsatellite markers. Their number of alleles per locus ranged from 2 to 9 with an average of 4.69 alleles per locus. Their PIC ranged between 0.24 and 0.956 with an average of 0.811 per locus, which were all higher than that reported in this work.

High PIC value of a marker indicates high probability to detect the number of alleles among cultivars. A PIC value higher than 0.50 indicates high degree of polymorphism. Based on this RM20, RM10864, RM10793, RM10748

and RM10722 were very good primers for this diversity study. The total number of alleles generated by the 28 primers agrees with findings of Zeng et al. (2004) who observed a total of 123 alleles among 33 rice genotypes with an average of 4.9 alleles per locus. The number of alleles per locus ranged from 2 to 9. The PIC values for the microsatellite loci ranged from 0.06 to 0.85 with an average of 0.57. Low PIC values were observed for 5 primers and the PIC values for the remaining 17 microsatellite loci were all above 0.50. Lapitan et al. (2007) also obtained PIC ranges from 0.18 to 0.91 with an average 0.68 per marker, making them very useful for genotypic studies. Prabakaran et al. (2010) had a total of 11 alleles detected by 5 SSR primers and the number of alleles per locus ranged from 2 to 3 with an average of 2.2 per locus. Among the primers used, RM 481 identified higher number of alleles and average PIC was 0.43. Behera et al. (2012) observed a wider range of PIC, between 0.24 and 0.956 with a higher average of 0.811 per locus than was obtained in this study. The results of this study show that the markers used are revealing and good for genetic diversity studies in rice. Microsatellites are efficient and cost-effective to use. Compared with other markers, they are abundant, co-dominant, highly reproducible and interspersed throughout the genome. In particular, microsatellite markers have been widely applied in rice genetic studies as they are able to detect high levels of allelic diversity. SSR markers are playing important role in identifying genes for salt tolerance that can be helpful for plant breeders to develop new cultivars. Molecular markers could be used to tag QTL and evaluate their contributions to the phenotype by selecting for favorable alleles at those loci in marker assisted selection (MAS) scheme with the aim to accelerate genetic advancement in rice. This is faster, more efficient and cost-efficient than conventional screening under saline field conditions (Gregorio, 1997; Aliyu et al., 2011). The findings in this study imply a great genetic resource for improvement to salinity of rice in Ghana. SSRs discovered here can be incorporated in breeding programs to improve rice materials for farmers.

Selection of salt tolerant genotypes

Progress in rice breeding for salt tolerance entails identifying the major locus with salt tolerance at different growth stages. Out of 14 saltol primers screened, only primers RM10711 and RM10793 were able to discriminate tolerant genotypes from susceptible ones. Based on Primer RM10711, Nerica L23, Local Red, IR 72 (Ph) were tolerant to salinity stress, CG14, Nerica L9, Nerica L24, Nerica L27, Sebota 33, Anyofula, Matigey, Basmati 122, GR 18 Red, Local Basmati-2, Koshihikari, Viwornor, Sebota 281-2, Sebota 68, GH 1593, GH 1575, GH1598, GH1571, GH 1533, GH 1599 were however sensitive to salinity stress. With regard to RM10793, GH1575, GH1585, GH1598, GH1528, GH1545, GH 1580, CG14, Nerica L23, Nerica L24, Sebota 33, Sebota 41, Local Red, Good and New (JP), IR 72 (Ph), Local Basmati-2, Koshihikari, Viwornor were tolerant and GH 1599, SR-1, GH1571, GH 1533, Nerica L9, Nerica L27, Perfume (Short type), GH 1837, Matigey, Basmati 122, Sebota 281-2, Sebota 68 were susceptible accessions. This indicates that the primers could be used in marker assisted selection involving these genotypes. Aliyu et al. (2011) used RM10793 on a collection of 150 diverse rice genotypes with a tolerant salt variety Pokkali and found the marker very informative. Deepti et al. (2013) also found the primer very informative in their study on salt tolerance in some rice accessions. Huyen et al. (2012) used RM10793, RM10711 in Introgressing salinity tolerance QTLs Saltol into AS996 rice variety with five hundred BC2F1 individuals. Kabir et al. (2008) also used twelve SSR markers for parental survey and among them three polymorphic SSR markers, OSR34, RM443 and RM169 were selected to evaluate 26 F3 rice lines for salt tolerance. With respect to marker OSR34, 15 lines were identified as salt tolerant, 9 lines were susceptible and 2 lines were heterozygous. Several SSR primers (RM21, RM51 and RM127) were used by Sohrawardy et al. (2008) for the identification of salt tolerant rice lines of PNR-519 x IR29 in F3 population. Islam et al. (2008) selected different SSR primers to evaluate F2/F3 rice lines for salt tolerance and identified 15 rice lines as salt tolerant by using RM231 and RM24 primers.

Clustering of genotypes

Dendrogram generated by SSR primers further grouped the germplasm into three major clusters. Cluster 1 had IR29 and FL478 clustering together; it also had the Nerica's at different sub clusters. Nerica L9 is a cross between TOG5681 and 3 rounds of backcross to IR64, Nerica L-23 and Nerica L-24 are crosses between TOG5681 and 2 rounds of backcross to IR64 followed by crosses to IR31851-96-2-3-2-1, Nerica L-27 crosses between TOG5681 and 4 rounds of backcross to IR64. This probably explains why they were in the first cluster together, even though at different sub clusters. FL478 is a salt tolerant variety developed from a cross between Pokkali and IR29. The genotypes that clustered closely to these checks were similar to them.

Major cluster 2 had GH1598, GH1585, GH1545, GH 1533, clustering with CG14, a glaberrima and native to Africa, at a lower clustering level. This means the varieties in this group could be indigenous landraces from Africa and may carry a lot of unexploited genes for rice breeding. The glaberrima carries genes for tolerance to a lot of natural stresses, from environmental to biotic stresses (Takeoka, 1965; Second, 1984). Abidjan, Koshihikari, Local Basmati-2 and GH 1599 also formed a cluster; Koshihikari is a known Japanese elite variety,

with cold tolerant genes and the Basmatis are known for their aroma. Sub clustering under this group showed SR-1 singly and separately clustering with the group, Sebota 68, Basmati 122, Sebota 281-2, Sebota 33, Matigey, and GH 1837. SR-1 seed shows shattering tendency when mature. This may imply that the group clustering with it could behave similarly. There is also the possibility that SR-1 is the only one with the shattering ability, hence on a different branch. Shattering is a negative trait in rice breeding. Major cluster 3 had only IR72 which had high amylose content; it is popular among Cambodian farmers because it produces higher yields with superb grains of good quality, long grain and good taste, and can grow in dry season too. This accession did not cluster with any of the accessions indicating how unique and diverse it was from the rest.

The germplasm from PGRRI showed high degree of variability indicating how widely diverse they are genetically and how rich the germplasm is. This is good for rice breeding as it indicates a rich array of genes which could be useful for improving the crop. These results further highlighted the divergence of the population studied. This diversity can be explored in breeding to improve local rice cultivars. Microsatellite markers were able to distinguish between salt tolerant and susceptible entries.

Conclusion

The high PIC value of RM20, RM10864, RM10793, RM10748 and RM10722 and their ability to separate the rice germplasm suggests their usefulness in diversity studies. Genotypes GR18Red, GH1580, GH1528, GH1575, Anyofula, Local Red and NericaL23, NericaL24 and NericaL27 were selected by the markers to be tolerant to salinity stress. They would therefore be candidate genotypes for further development of improved varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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