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Assessment of Genetic Biodiversity of Several Traits Using SSR Markers in Rice (*Oryza sativa* L.)

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Abstract

Eight primers (RM 315, RM 318, RM 166, RM 302, RM 201, RM 234, RM 526 and RM 144) revealed different levels of polymorphism to tag the related traits of interest as tolerant to abiotic stress, resistant to biotic stress and yield-related traits. Two primers (RM 190 and RM 278) were monomorphic. The percentage of the polymorphism was nearly 80 %. The size of detected fragments ranged from 105–325 bp. A total of 186 bands were scored from the amplification products with the ten SSR primers. Genetic diversity analyses were conducted on the basis of the scores with 176 unique bands. Phylogenic tree for the fifteen rice accessions from each group were established according to the molecular data and based on ten SSRs. A marked genetic diversity was observed in these innovative accessions (Sakha 101, IR 03N137, IR 83142-12, IR 87856-10-AJY-1-B, HHZ 12-Y4-DT1-Y2 and IR 1552), which revealed higher levels of diversity and hence can be used as donors for the effective conservation, utilization and providing favorable genes in rice breeding programs.

Keywords: Rice, Genotypes, Biodiversity, Evolution, SSRs.

Introduction

In a set of ninety rice accessions divided into five groups were evaluated under normal and stress conditions. This work was divided into two parts: 1) Assessment of genetic biodiversity, which include phenotypic evaluation, genetic parameters, correlation coefficient estimation and morphological clustering for all the studied accessions. 2) Estimation of the genetic structure and divergence by utilizing marker assisted selection (MAS), SSRs to tag favorable quantitative trait loci, molecular profiling for establishing phylogenic tree to detect the origin/evolution among all the tested accessions.

Assessment and estimation of genetic biodiversity and the discovery of evolved genotypes through the breeding process can bring a new point of view (Thomson et al., 2007). For finding out knowledge of multiple facets about rice and considering genetic diversity from phenotype to molecule which is essential for the effective conservation, utilization and providing favorable genes and valuable germplasm to be integrated into new varieties are vital to achieve advanced breeding.

Genetic biodiversity in a set of germplasm is very important for identifying new genes and for improvement of the germplasm. For this, many efforts have been made to assess the genetic diversity and relationships among germplasm collections of rice using DNA markers. Conserving agricultural diversity and assessment at the genetic level is a prerequisite for understanding the evolutionary patterns and developing suitable

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strategies for their conservation and sustainable use (Kumar et al., 2010).

Genetic relationship estimates based on data on the frequencies of alleles at specific microsatellite loci indicated that the majority of traditional varieties were poorly related to the improved varieties (Alvarez et al., 2007). Then, using SSRs markers which are simple tandemly repeated 5-20 fold; often ditto tetra-nucleotide; sequence motifs; each flanked by unique sequences, (IRGSP, 2005). These markers are valuable as genetic markers because: they are co-dominant in nature; show high polymorphism; are cost effective and easy to use in PCR; and used in automated genotyping.

Tens of thousands of potential SSRs have been identified in rice, and over 25,000 have been developed as molecular markers which are currently being used to develop high density genetic maps, genotype rice accessions, determine the genetic structure, optimize the assembly of core collections, and for marker-assisted selection in breeding programs.

The accessible genetic biodiversity whether natural or induced is a prerequisite for understanding the biological and evolutionary process of a particular set of rice accessions represented in a given geographical region or during the long-term domestication and cultivation under various ecological niches (IRRI, 2010). Rice accessions that are highly with divergent genetic background may help to speed up the breeding process to reach the status of new lines through proper crosses and backcrosses (Ferrero A, Vidotto F. 2010). Rice accessions with diverse genetic relationships will be used as innovative donors (e.g., resistant to biotic and tolerant to abiotic stresses) and donors for the effective conservation, utilization and providing favorable genes in rice breeding programs.

Materials and Methods

Plant Materials

Seeds of all the studied rice accessions were obtained from Rice Research and Training Center (RRTC) and from The International Network for Genetic and Evaluation of Rice (INGER) based at the International Rice Research Institute (IRRI). Genomic DNA was isolated from ninety rice accessions using CTAB (Cetyl-tetramethyl ammonium bromide), as described by Murray and Thompson, 1980.

PCR amplification of SSR markers was carried out using ten primers and were carried out in 10 µl volume., Accessions and primers are shown in tables 1 and 2, respectively. The accessions divided into five groups:

1. Cultivated varieties and promising lines: consisted of fifteen cultivated varieties and promising lines for rice production.
2. Conservation group: were divided into two subgroups (ex-situ & in-situ)

2.A. Irrigated rice (ex-situ): consisted of fifteen early-maturing accessions

2.B. Upland rice (in-situ): contained fifteen genetically-diverse test accessions.

3. Soil stress tolerance: consisted of fifteen accessions for salinity tolerance
4. Green super rice: consisted of fifteen accessions for rice production
5. Blast resistance: consisted of fifteen blast monogenic lines for blast resistance.

The experiment was set in a randomized complete block design with 3 replications. For each accession, 20 seeds were planted in a greenhouse and leaves from 20-day old seedlings were collected for DNA extraction.

Molecular Analysis

DNA Isolation and Quantification

Total genomic DNA were isolated from young leaves grown in the green house for 15 days using CTAB method Muray and Tompson, 1980, quantification was carried out by gel based assay using different concentrations of λ un-cut DNA.

SSRs Based on Polymerase Chain Reactions

PCR reactions for SSR markers were carried out in 10 ul volume containing 1.0 µl total genomic DNA 4.9 ul H2O, 1.0 µl 10 X PCR buffer, 0.8 µl Mg Cl2, 0.4 µl dNTPs, 0.3 µl Taq polymerase and 0.3 µl from SSR markers (forward and reverse primers). Amplification was performed in Berkin Elemar Gene Amp PCR system 2400 and DNA Engine Peltier Thermal Circler with following the profile, 950C for 5 min (initial denaturation), 950C for 1 min, 550C for 1 min, 720C for 2 min and for 35 cycles with final extension 720C for 7 min. was established, followed by storage at 4°C. PCR thermocycler machines from Biometra and Applied Bio Systems were used (Chen et al., 2006).

Ten simple sequence repeats (SSR) primers were used according to the trait of interest in each group and subgroup,: RM 190 for thousand grain weight trait in group I. RM 315 for plant height trait and RM 318 for panicle length trait in subgroup II.A., RM 166 and RM 302 for drought tolerance trait in subgroup II.B., RM 201 and RM 234 for salinity tolerance trait in group III. RM 278 and RM 526 for grain yield related traits in group IV. and RM 144 for blast resistance trait in group V. These primers sequences were as shown in Table 2.

The PCR products were analyzed directly on 1.5 % agarose gels in 0.5x TAE buffer, visualized by staining with ethidium bromide and Transillumination under ultra violet light. Comparison of accessions, based on the presence (1) or absence (0) of unique and shared polymorphic products was used to generate similarity coefficients using UPGMA method with statistical software package NTSYSpc2.1 (Rohlf, 2001).

The Polymorphism Information Content (PIC) value was calculated using the following formula: $PIC_i=1-\sum(Pij)2$. Where Pi is the proportion of samples carrying the allele of a particular locus, Pij is the frequency of jth allele for i marker and the is for final score of all alleles for the given marker, (Botstein et al., 1980; Anderson et al., 1993).

Table 1. Rice accessions for all five groups.

No.	Entry Name	Cross	Origin
		Group I.	
1	Sakha 101	Milyang 79/Giza 176	RRTC
2	Sakha 104	GZ 4100/GZ 4096	RRTC
3	Giza 177	Pi No.4// Yumji No.1 /Giza 171	RRTC
4	GZ 10154-3-1-1-1	Sakha 101/Sakha 105	RRTC
5	GZ 9399-4-1-1-2-1-2	IR 65600/Giza 178	RRTC
6	Sakha 105	GZ 4316/GZ 5581	RRTC
7	Sakha 106	Hexi 30/Giza 177	RRTC
8	Sakha 102	GZ 4120/GZ 4096	RRTC
9	GZ 10147-1-2-1-1	IRI 385/GZ 6124-4-1-1-1	RRTC
10	GZ 9399-4-1-1-3-2-2	IR 65600/Giza 178	RRTC
11	Giza 182	Giza 181// IR 39422 – 163 -1-2 /Giza 181	RRTC
12	GZ 9461-1-4-2-3-1	Dacw 2 Bayo/GZ 6296	RRTC
13	GZ 9807-6-3-2-1	Yunlen 19/GZ 7102 -20-82-1	RRTC
14	Egyptian Jasmine	KDML 105/IR 262-43-8-11	RRTC
15	GZ 10101-5-1-1	IRI 385/Sakha 103	RRTC
		Group II. Subgroup II.A.	
1	IR 03N137	IRRI 134/IR 70479-45-2-3//IR 64680-81-2-2-1-3	IRRI
2	HHZ 1-Y4-Y1	HUANG-HUA-ZHAN* 2/YUE-XIANG-ZHAN	IRRI
3	IR 02A127	IR00A107/IR 62243-41-1-3-3	IRRI
4	IR 06M147	MEM BERANO/PADI ABANG GOGO	IRRI
5	IR 10N108	IR 65620-192-3-3-3-2/IR02N463//IR 72875-94-3-3-2	IRRI
6	IR 07A234	NSIC RC 138/IRRI 123	IRRI
7	HHZ 12-DT 10-SAL 1-DT 1	HUANG-HUA-ZHAN* 2/TE QING	IRRI
8	RI 1812084-8-1-1	NEAMAT/IR 67014-138-3	IRAN
9	IR 06A181	IR 71718-59-1-2-3/IR 72	IRRI
10	IR 06M143	MEM BERANO/PADI ABANG GOGO	IRRI
11	HHZ 8-SAL6-SAL3-Y2	HUANG-HUA-ZHAN* 2/PHALGUNA	IRRI
12	IR 05N412	IR 72875-94-3-3-2/IR 73707-45-3-2-3	IRRI
13	IR 09N500	IR 66/IRRI 146	IRRI
14	BP 10620F-BB4-15-BB8	CIHERANG/IR BB 64	INDONESIA
15	IR 06N119	IR 73707-45-3-2-3/IR 68552-100-1-2-2	IRRI
		Group II. Subgroup II.B.	
1	IR 83142-12	IR 06G103/IR 06G113	IRRI
2	IR 60080-46A	IR 47686-08-4-3/CT 6516-21-4-4	IRRI
3	IR 08L152	IR 78875-176-B-2/IR 78875-207-B-3	IRRI
4	IR 10L240	IRRI 132/IR06L161	IRRI
5	IR 10L107	IR 70181-32-PMI 1-1-5-1/IR 71700-247-1-1	IRRI
6	IR 11L319	IR06L142/IR11L101	IRRI
7	IR 11L290	IRRI 119/BP 234 E-MR-11	IRRI
8	IR 11L276	IRRI 119/IR11L101	IRRI
9	IR 10L411	IR 78878-53-2-2-2/IR03A550	IRRI
10	IR 09L348	IRRI 105/IR 78877-163-B-1-1	IRRI
11	IR 10L398	IR06L161/IR 80508-B-57-3-B	IRRI
12	IR 08L161	IR 78875-176-B-2/IR 78875-207-B-3	IRRI
13	IR 11L301	IR06L142/IR11L101	IRRI
14	IR 09L179	IR 78878-53-2-2-2/CT 6510-24-1-2	IRRI
15	IR 09L173	IR 78877-208-B-1-2/IRRI 148	IRRI

Table 1. Continued.

		Group III.	
1	IR 87856-10-AJY 1-B	AT 401/IR 03A550	IRRI
2	IR 87938-1-1-1-3-B	IR 4630-22-2-5-1-3/IR05N204	IRRI
3	IR 87938-1-1-2-2-B	IR 4630-22-2-5-1-3/IR05N204	IRRI
4	IR 87938-1-1-2-3-B	IR 4630-22-2-5-1-3/IR05N204	IRRI
5	IR 88300-2-2-1-B	IRRI 123/IR 66946-3R-178-1-1//A 69-1	IRRI
6	IR 86376-47-3-1-B	IR07F101/IRRI 126	IRRI
7	AT 401	BG 94-1/POKKALI	SRI LANKA
8	NONA BOKRA	-	INDIA
9	POKKALI (ACC108921)	POKKALI	INDIA
10	IR 85920-11-2-1-AJY1-3-B	IR 84087-19/IR00A110//IR00A110	IRRI
11	IR 85921-9-2-1-AJY1-1-B	IR 84087-29/IR00A110//IR00A110	IRRI
12	IR 87856-7-AJY1-B	AT 401/IR03A550	IRRI
13	IR 87938-1-1-1-2-B	IR 4630-22-2-5-1-3/IR05N204	IRRI
14	IR 88304-B-AJY1-B	IRRI 126/IRRI 135//IR 66946-3R-156-1-1///IR 55179-3B-11-3	IRRI
15	IR 88314-1-AJY1-B	IR 73718-23-2-1-3*2/IR 66946-3R-178-1-1	IRRI
		Group IV.	
1	HHZ 12-Y4-DT1-Y2	HUANG-HUA-ZHAN*2/TE QING	IRRI
2	HHZ 8-SAL9-DT2-Y2	HUANG-HUA-ZHAN*2/PHALGUNA	IRRI
3	KCD 1	-	CHINA
4	HHZ 11-Y6-Y2-SUB1	HUANG-HUA-ZHAN*2/IR 64	IRRI
5	HHZ 10-DT7-Y1	HUANG-HUA-ZHAN*2/ZHONG 413	IRRI
6	HHZ 12-Y4-DT1-Y3	HUANG-HUA-ZHAN*2/TE QING	IRRI
7	HUA 564	-	CHINA
8	HHZ 5-Y4-SAL1-Y1	HUANG-HUA-ZHAN*2/OM 1723	IRRI
9	HHZ 12-SAL2-Y3-Y2	HUANG-HUA-ZHAN*2/TE QING	IRRI
10	HHZ 5-SAL10-DT3-Y2	HUANG-HUA-ZHAN*2/OM 1723	IRRI
11	HHZ 12-SAL8-Y1-Y2	HUANG-HUA-ZHAN*2/TE QING	IRRI
12	HHZ 12-SAL8-Y1-SAL1	HUANG-HUA-ZHAN*2/TE QING	IRRI
13	HHZ 5-DT7-Y3-SAL1	HUANG-HUA-ZHAN*2/OM 1723	IRRI
14	HHZ 5-DT20-DT3-Y2	HUANG-HUA-ZHAN*2/OM 1723	IRRI
15	HHZ 8-SAL14-SAL3-Y2	HUANG-HUA-ZHAN*2/PHALGUNA	IRRI
		Group V.	
1	IR 1552	IR 160-25-1-1/CROSA 2	IRRI
2	B 40	KAOHSIUNG 21//SERATUS MALAM/IR5	IRRI
3	IRRI 123	IR 47761-27-1-3-6/IR 56381-139-2-2	IRRI
4	IR 78581-12-3-2-2	IR 73012-137-2-2-2/PSB RC 10	IRRI
5	IR 11A208	IR00A107/IR 72875-94-3-3-2	IRRI
6	IR 10A234	IR04A115/IR 72875-94-3-3-2//IRRI 135	IRRI
7	IR 10A231	IRRI 143/IR 73718-23-2-1-3//IR00A110	IRRI
8	IR 10A228	IR01A154/IR 72870-19-2-2-3//IRRI 123	IRRI
9	IR 10A227	IR01A154/IR 72870-19-2-2-3//IRRI 123	IRRI
10	IR 10N290	IR05N341/IR 64680-81-2-2-1-3	IRRI
11	IR 10A196	IR04A285/IRRI 123	IRRI
12	IR 10N253	IR BB 60-1/IR 73711-130-1-3-1//IR 65450-173-2-1-1-3-3	IRRI
13	IR 10N205	IR 72906-32-1-3-3/IR 73712-68-3-1-2//IR 65450-173-2-1-1-3	IRRI
14	IR 12M101	IR 64/IR 68144-2B-2-2-3-1-166	IRRI
15	IR 10A121	IR 72906-32-1-3-3/IR 72890-81-3-2	IRRI

Table 2. Forward and reverse sequences of ten SSR primers.

Primer	Forward sequence	Reverse sequence
RM190	5¯ ctttgtctatctcaagacac 3¯	5¯ ttgcagatgttcttcctgatg 3¯
RM 315	5¯ gaggtacttcctccgtttcac 3¯	5¯ agtcagctcactgtgcagtg 3¯
RM 318	5¯ gtacggaaaacatggtaggaag 3¯	5¯ tcgagggaaggatctggtc 3¯
RM 166	5¯ ggtcctgggtcaataattgggttacc 3¯	5¯ ttgctgcatgatcctaaaccgg 3¯
RM 302	5¯ tcatgtcatctaccatcacac 3¯	5¯ atggagaagatggaatacttgc 3¯
RM 201	5¯ctcgtttattacctacagtacc3¯	5¯ ctacctcctttctagaccgata 3¯
RM 234	5¯ acagtatccaaggccctgg 3¯	5¯ acgtgagacaaagacggag 3¯
RM 278	5¯ gtagtgagcctaacaataatc 3¯	5¯ tcaactcagcatctctgtcc 3¯
RM 526	5¯ cccaagcaatacgtccctag 3¯	5¯ acctggtcatgacaaggagg 3¯
RM 144	5¯ tgccctggcgcaaatttgatcc 3¯	5¯gctagaggagatcagatggtagtgcatg 3¯

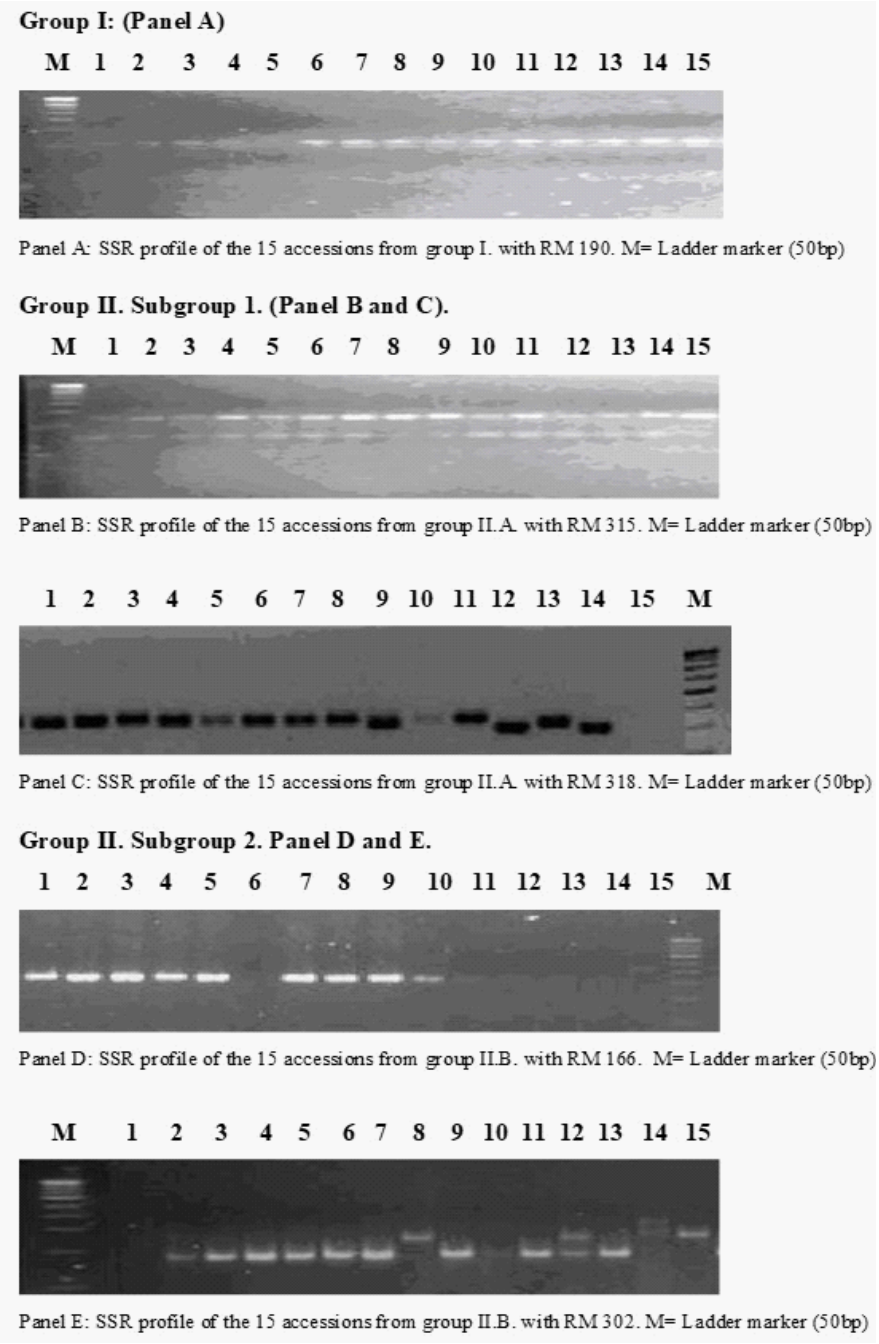
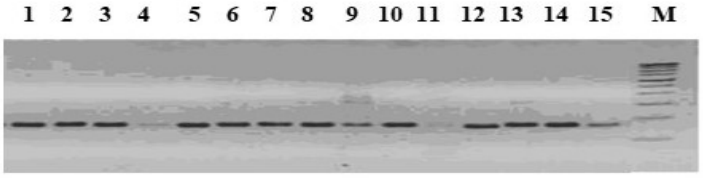
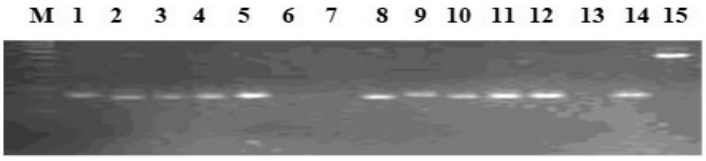


Figure 1. Panels A - J.

Group III. (Panel F and G)

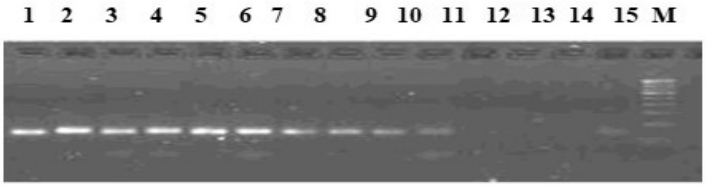


Panel F: SSR profile of the 15 accessions from group III. with RM 201. M= Ladder marker (50bp)

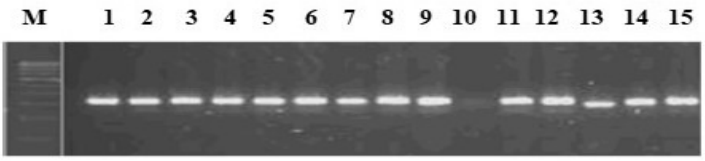


Panel G: SSR profile of the 15 accessions from group III. with RM 234. M= Ladder marker (50bp)

Group IV: Panel (H and I).

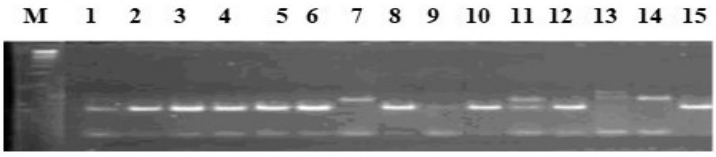


Panel H: SSR profile of the 15 accessions from group IV. with RM 278. M= Ladder marker (50bp)



Panel I: SSR profile of the 15 accessions from group IV. with RM 526. M= Ladder marker (50bp)

Group V: (Panel J).



Panel J: SSR profile of the 15 accessions from group V. with RM 144. M= Ladder marker (50bp)

Figure 1. Continued.

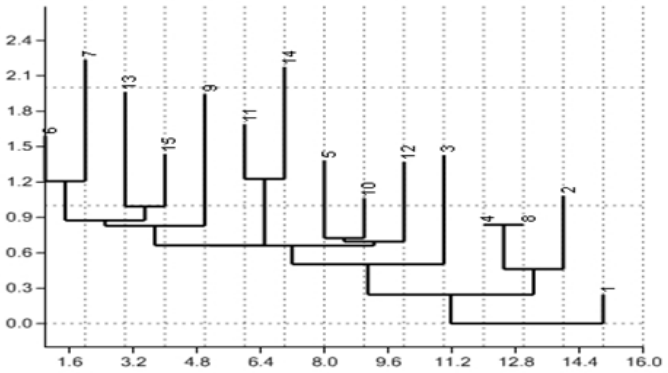


Figure 2. UPGMA dendrogram showing the phylogenic relationships among the accessions based on SSR markers.

Table 3. Scoring data of 15 rice genotypes from each group against 10 SSRs.

RM 190	RM 315	RM 318	RM 166	RM 302	RM 201	RM 234	RM 278	RM 526	RM 144
	Group	Group	Group	Group	Group.	Group.	Group.	Group.	Group.
Group 1	2. A.	2. A.	2. B.	2. B.	3	3	4	4	5
1	1	1	1	1	2	1	1	1	1
1	1	1	1	1	2	2	1	1	1
1	2	1	1	1	1	2	1	2	1
1	1	1	1	1	2	2	1	2	1
1	2	1	1	1	2	1	1	2	1
1	1	1	1	1	2	0	1	2	1
1	1	1	1	2	2	0	1	2	2
1	1	1	1	1	2	2	1	2	1
1	1	1	1	1	2	1	1	3	0
1	2	1	1	1	2	1	1	2	1
1	2	1	1	0	2	1	1	2	2
1	2	1	0	1	2	1	1	2	1
1	2	1	1	0	2	0	1	2	0
1	2	2	1	0	2	1	1	3	2
1	2	1	1	1	2	0	1	2	1

Results and Discusson

Molecular Profiling for the Rice Accessions

Ten primer pairs flanking simple sequence repeats which were used to determine the level of polymorphism among fifteen rice accessions in each tested group and subgroup, as shown in Figure 1 (Panels A-J). Eight primers (RM 315, RM 318, RM 166, RM 302, RM 201, RM 234, RM 526 and RM 144) were polymorphic to tag the related traits of interest as tolerant to abiotic, resistant to biotic stresses and grain yield. While, two primers (RM 190 and RM 278) were monomorphic. In group I: RM 190 was used for detecting major quantitative trait loci associated with thousand grain weight trait in the best selected accessions according to its morphological data. Only one fragment was detected in the range from 105 to 125 bp as shown in Figure 1 (Panel A) and was monomorphic for this trait.

Regarding group II. - subgroup II.A. RM 315 and RM 318 primers which were used for detecting major quantitative trait loci associated with plant height and panicle length traits, respectively in the best selected accessions. For RM 315, a DNA fragment was generated with molecular size from 130 to 160 bp. monomorphic for plant height trait, as shown in Figure 1 (Panel B). RM 318 was used for observing major quantitative trait loci associated with panicle length trait and revealed polymorphic variation with fragment size ranged from 135 to 155 bp as shown in Figure 1 (Panel C).

DNA in in rice accessions for group II. - subgroup II.B. was amplified with RM 166 and RM 302 were used for observing major quantitative trait loci fragments associated with drought tolerance trait. RM 166 and RM 302 showed polymorphic variations with fragment size from 320 to 325 bp. and from 120 to 190 bp as shown in Figure 1 (Panels D and E). RM 201 and RM 234 were used to tag major quantitative trait loci fragments related to saline tolerance trait in group III. DNA amplified two fragments with RM 201 and RM 234 which revealed polymorphic variations with the first fragment size which was ranged from 145 to 160 bp while the second fragment was ranged from 130 to 165 bp as shown in Figure 1 (Panels F and G).

In group IV: RM 278 and RM 526 amplified two different fragments with size range from 130 to 145 bp and from 240 to 266 bp., to tag major quantitative trait loci fragments associated with yielding in the fifteen rice accessions. RM 278 was monomorphic, while RM 526 revealed polymorphic variation as shown in Figure 1 (Panels H and I).

Considering group V: DNA from the fifteen rice accessions amplified with RM 144 to tag major quantitative trait loci associated with blast resistance trait. Amplified DNA fragments with RM 144 revealed polymorphic variations and was observed with size range from 215 to 255 bp as shown in Figure 1 (Panel J).

Drive to conclusion that there was polymorphism to reveal diversity among accessions and validate known linked markers. Also, it may suggest a linkage between the chosen markers and valuable traits. These results agreed with those published earlier by (Islam et al., 2012; Lin et al., 2012).

Estimation of Polymorphic Information Content

Genetic polymorphism among rice accessions was established by using ten (SSR) Markers Seetharam et al., (2009) which recorded high level of polymorphism (80%) number of alleles detected were for tagging major quantitative trait loci fragments associated with traits of interest as tolerant to abiotic, resistant to biotic stresses and grain yielding. Alleles ranged from 1 in case of RM 166 to 3 in case of RM 144). A total of 186 bands were scored from the amplification products with ten SSR primers. Genetic diversity analyses were conducted on the basis of the scores with 176 unique bands, as shown in Table 3. The large range of polymorphism values for related accessions using SSRs provide greater confidence for the assessment of genetic diversity Meti et al. (2013).

Out of ten primers of SSR, eight primers were found to be polymorphic and Polymorphic information content (PIC) value was 0.827. Polymorphism in rice accessions offer unique opportunity to study phylogenitic relationships and evolutionary comparisons and suggested that those markers that were highly

variable at both the inter-subspecific and intra-subspecific characteristic and molecular levels, are very useful for distinguishing closely related accessions. These results agreed with those obtained previously by Das et al., (2013). These results agreed with those published earlier by Pachauri et al. 2013. It can be inferred from the result that a significant genetic diversity since high polymorphism has been detected among all accessions and thus there is a good possibility of genetic improvement using such a set of genotypes in rice breeding program.

Phylogenic Relationships Among the Accessions Based on SSR Markers

The phylogenic tree of fifteen rice accessions of each group and subgroup were established according to molecular data based on ten SSRs markers and constructed by Neighbor joining tree Cai et al., (2013). Rice accessions were clustered based on their banding patterns using UPGMA method largely on their genetic background and (Zhuoxian et al., 2014). The dendrogram is shown in Figure (2), which resulting from UPGMA analysis showed clusters of various sizes, Qianjin et al., (2006).

In group I. all fifteen accessions were diversified into various clusters, Sakha 101 was diversified earlier and was suggested to be an origin for the cultivated varieties and promising lines in this group. Two clusters were grouped into, X1 consisted of (Sakha 104, Sakha 102 and Gz 1015-3-1-1-1) while the cluster X2 consisted of eleven rice accessions grouped into four subclusters with (Giza 177) as out branch. Therefore, the latter supposed to be an origin for cluster X2. Sakha 105 and Sakha 106 diversified recently and these two accessions which actually have been used as new cultivated varieties.

Regarding group II. - subgroup II. A. IR 03N137 represented the origin for two clusters, X1 consisted of (HHZ 1-Y4-Y1, RI 1812084-8-1-1and IR 06M150), while X2 was divided into four sub-clusters with IR 02A127 accession as earlier diversified origin. while, subgroup II. B. the accession IR 83142-12 was diversified as an origin, X1 consisted of (IR 60080-46A, IR 11 L 276 and IR 10L240), while X2 consisted of four subclusters and accession IR 08L152 was diversified as an origin for cluster X2. Considering group. III., the accession IR 87856-10-AJY-1-B was revealed as an origin and the rest of the fifteen accessions were clustered into two clusters, X1 contained rice accessions (IR 87938-1-1-1-3-B, NONA BOKRA and IR 87938-1-1-2-3-B), while X2 consisted of four subclusters with IR 87938-1-1-2-2-B as out branch and supposed origin for cluster X2. In group IV. The accession HHZ 12-Y4-DT1-Y2 was separated earlier as an origin, cluster X1 consisted of three accessions (HHZ 8-SAL9-DT2-Y2, HHZ 5-Y4-SAL1-Y1 and HHZ 11-Y6-Y2-SUB1) while cluster X2 consisted of eleven rice accessions grouped into four sub-clusters. In group V. the accession IR 1552 was diversified earlier as an origin and lead to two clusters; cluster X1 with three accessions which were (B 40, IR 10A228, and IR 78581-12-3-2-2) while cluster X2 grouped into four sub-clusters with IRRI 123 as out branch. Sakha 101, IR 03N137, IR 83142-12, IR 87856-10-AJY-1-B, HHZ 12-Y4-DT1-Y2 and IR 1552 revealed higher levels of diversity and hence showed their ability to be used for effective conservation, utilization and providing favorable genes and valuable germplasm for rice breeding. Morphological and molecular variations are very important since rice genotypes are highly domesticated. In fact, domestication process is accompanied by genetic erosion, which causes a reduction in genetic diversity among traditional varieties and gradual loss of genotypes, (Wang et al., 2014). Morphological and genetic diversity have occurred in rice during evolutionary process and residual overlaps of key genetic differences in different genotypes from different regions exit. SSR can therefore be used as remarkable tools to detect genetic diversity for breeding programs of rice.

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