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ORIGINAL ARTICLE

Assessment of ISSR based molecular genetic diversity of Hassawi rice in Saudi Arabia

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Abstract Inter simple sequence repeat (ISSR) analysis, using 14 primers was performed to estimate genetic diversity among 27 landraces of Hassawi rice growing in Al-Ahsa region of Saudi Arabia and deposited at King Abdulaziz City for Science and Technology with KACST IDs. The average polymorphism produced by 11 selected primers was more than 75%. The analysis of ISSR polymorphism divided the examined rice landraces into two groups; In one group (A), one accession (KACST 191) was clearly delimited as a distant landrace from other 12 landraces grouped in two clusters; cluster I of seven landraces of close geographic distributions; four of them grow at close geographic locations (KACST IDs 32, 183, 184, 185, 186, 187 and 188) and cluster II is comprised of five landraces KACST IDs (190, 308, 352, 353 and 355). In group B, the landraces were more closely related to each other as compared to the landraces of group A. In this group a small cluster of two landraces (KACST 305 & KACST 333) was clearly distant from a large group of three clusters comprised of landraces having KACST IDs 189 & 192, landraces 302, 306, 307, 308 & 310 and landraces with KACST IDs 334, 351, 354, 356 & 357 respectively. These results indicate that ISSR fingerprints are efficient in the identification and resolution of genetic diversity between the landraces of the Hassawi rice and will be an efficient method in the authentication of the rice germplasm in the gene bank of Saudi Arabia.

1. Introduction

Rice (*Oryza sativa* L.) is one of the three ergonomically most important cereal crops and is serving as a staple food for over half of the world's population. The different varieties of rice are not considered interchangeable, either in food preparation or agriculture. Molina et al. (2011) reported evidence that rice originated from a single domestication origin around 8200–13,500 years before present (ybp), in the Yangtze Valley of China (Huang et al., 2012). There is ample evidence that in

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old days, rice was also grown in the Middle East particularly in areas of southern Iraq. Its cultivation was also spread to Iran with the advent of Islam ca 1400 ybp. In Egypt, rice is mainly grown in the Nile Delta and in Saudi Arabia in Al-Ahsa region east of the country and is therefore known as Al-Hassawi or Hassawi rice.

The Hassawi rice is a landrace adapted to the climate of Eastern Saudi Arabia and characterized by strong adaptability to soil salinity and drought. However, it bears some undesired characteristics such as susceptibility for lodging, delayed maturity, and photoperiod sensitivity, it is planted after the summer months; usually in September and October (Al-Mssallem and Al-Mssallem, 1997). Hassawi rice is eaten in its whole form, which means the outer bran layers are not removed. It is used traditionally to provide strength for those who are unwell. Rice growing is expensive given the lack of farmers, the difficulty in growing rice in arid environment, and also the long growing season. When compared with Uncle Ben's rice, Hassawi rice is higher in protein, but lower in terms of both rapidly-available glucose (RAG) and slowly available glucose (SAG), although the GI was comparable with that of Uncle Ben's rice (Al-Mssallem et al., 2011).

There is a limited literature on the genetic background of Hassawi rice. Zhang et al. (2012) reported the presence of two cultivars of Hassawi rice in Al-Ahsa region. Hashawi-1 is the wild-type and originated from an indica ancestor while cultivar Hashawi-2 is a hybrid between Hashawi-1 and IR1112 (IRRI). IR1112 has maternal parent IR262-43-8-11, a cultivar originated from an Indonesian indica variety called "Peta". Frequent DNA rearrangement in the Hassawi mitochondrial (mt) and chloroplast (cp) genomes indicates ongoing dynamic processes to reach genetic stability under strong environmental pressures. Based on sequence variation analysis and the breeding history, Zhang et al. (2012) suggested that both Hassawi-1 and Hassawi-2 originated from the Indonesian variety Peta since genetic diversity between the two Hassawi cultivars is very low albeit an unknown historic origin of the wild-type Hassawi rice.

Inter-simple sequence repeats ISSR are a class of molecular markers based on inter-tandem repeats of short DNA sequences. These regions lie within the microsatellite repeats and offer great potential to determine intra-genomic and inter-genomic diversity compared to other arbitrary primers, since they reveal variation within unique regions of the genome at several loci simultaneously. They exhibit specificity of sequence-tagged-site markers, but need no sequence information for primer synthesis enjoying the advantage of random markers (Zietkiewicz et al., 1994; Goodwin et al., 1997). The primers used in ISSR analysis can be based on any of the SSR motifs (di-, tri-, tetra- or penta-nucleotides) found at microsatellite loci, giving a wide array of possible amplification products, and can be anchored to genomic sequences making either side of the targeted simple sequence repeats (Zietkiewicz et al., 1994). The ISSR method was proven especially useful in the Poaceae family for the analysis of nearly isogenic lines (Akagi et al., 1996) and in differentiation of rice varieties (Parsons et al., 1997). The ISSR markers based on AG, GA and (GATA)*n* repeats have been reported to be very informative and cost-effective in determining genetic relationships among diverse accessions of rice germplasm (Joshi et al., 2000; Davierwala et al., 2000; Sarla et al., 2003, 2005; Reddy et al., 2009).

In the present study, ISSR markers have been used in determining genetic diversity among different landraces of Hassawi rice. The efficiency of ISSR primers of di- and tri-nucleotide repeats has been tested in producing polymorphic DNA bands in the rice genome. Knowledge of the genetic diversity between landraces is useful for authentication of rice genotypes in Saudi Arabia and important for conservation of landraces in the gene bank. The conserved and authenticated materials are important sources for selecting superior, yet genetically divergent parents to optimize genetic variation in subsequent breeding programs.

2. Materials and methods

2.1. Plant material

Twenty seven accessions of Hassawi rice landraces were collected from farmers in Al-Ahsa region south east of the Kingdom of Saudi Arabia. The collected material was given permanent KACST IDs and deposited in the germplasm of King Abduaziz City for Science and Technology (KACST). For convenience, the accessions were numbered 1–27 as given in Table 1. Grains of the examined landraces were germinated on vermiculite in the green house and young seedlings were harvested and packed separately in polyethylene bags, immediately placed in ice box and transferred to the laboratory and stored at −70 °C until used for DNA extraction and ISSR fingerprinting.

Table 1 List of the KACST IDs for the 27 Hassawi rice landraces (accessions) used in the current study and their sites in Al-Ahsa region of Saudi Arabia.

Serial	KACST ID number	Site of collection and cultivation
01	KACST 32	Al-Atik farm, collected accession
02	KACST 183	Al-Atik farm, area 1
03	KACST 184	Al-Atik farm, field 1, plot 1
04	KACST 185	Al-Atik farm, field 2, plot 1
05	KACST 186	Al-Atik farm, field 2, plot 2
06	KACST 187	Al-Atik farm, field 2, plot 2
07	KACST 188	Al-Atik farm, area 2
08	KACST 189	Zaki Al-Salem farm
09	KACST 190	Habib Al-Attar farm
10	KACST 191	Abdul-Galil Al-Naghani farm
11	KACST 192	Moussa Al-Salem farm
12	KACST 302	Al-Atik farm, north, plot 1
13	KACST 305	Al-Atik farm, south
14	KACST 306	Al-Rashed farm
15	KACST 307	Yousef Al-Naser farm
16	KACST 308	Soliman Al-Mutawaei farm
17	KACST 309	Al-Atik farm, north, plot 3
18	KACST 310	Saleh Abdel-Kader farm
19	KACST 333	Al-Atik farm, south, plot 3
20	KACST 334	Al-Shieba, Atik, north
21	KACST 351	Al-Qarena, Hussain Ali farm
22	KACST 352	Al-Jalila, Sayed Ali farm
23	KACST 353	Al-Battalia, Ali El-Sheikh farm
24	KACST 354	Um Sabaa, Saleh Abdel-Kader farm
25	KACST 355	Al-Ghoba, Ahmad Al-Sheieb farm
26	KACST 356	Al-Ghoba, Adnan Al-Salem farm
27	KACST 357	Al-Nozha, Al-Rashed farm

2.2. Genomic DNA extraction

Different protocols were used for the extraction of the genomic DNA from the rice leaves and ultimately obtained the best results with the method of Dellaporta et al. (1983) with some modifications. In this protocol proteinase K treatment was used to inactivate the tissue nucleases. Fresh young leaves yielded DNA of a good quality and high quantity. The extracts were colorless and could be looped out easily. The average yields from 300 to 5000 mg of the leaves were 10–30 mg ml^{-1} DNA. A single thick band for each variety on the gel indicated a good quality and quantity of the DNA. The amount of DNA was estimated using a fluorometer (Hoefer DyNA Quant 200; Pharmacia Biotech, Piscatawaym, N.J.). Total genomic DNA was also run on a 1% agarose gel to determine the consistency and purity (Fig. 1). The stock DNA accessions were diluted with sterile TE buffer to make a working solution of 10 ng ml^{-1} for use in PCR analysis.

2.3. ISSR fingerprinting

A total of 20 ISSR primers were used for PCR amplification of the DNA templates. ISSR primers synthesized by 'IDT, Integrated DNA technologies' were used for PCR. The primers were dissolved in sterilized distilled water at a concentration of 10 pmol/µl. Amplification reactions were performed in volumes of 25 µl using "Ready-To-Go PCR Beads" kit (GE Healthcare Life Sciences) optimized for PCR reactions and contain thermo-stable polymerases (2.5 Units of recombinant puReTaq DNA polymerase), dNTPs (200 µM each dNTP in 25 µl reaction volume) and buffer [1.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris, (pH 9)] in a 25 µl reaction volume. In each reaction 25 ng of DNA accessions was used along with 20 pmol/µl of primer. PCR amplification was performed in Eppendorf Master Cycler Gradient PCR machine. The following PCR program was used: 5 min at 95 °C; followed by 35 cycles at 94 °C for 1 min, 45–55 °C (depending on the Tm of the primers) for 1 min and 72 °C for 2 min; then left at 72 °C for 10 min, followed by soaking at 4 °C.

The ISSR products were separated by electrophoresis according to their molecular weight on 1.4% (w/w) agarose gels submerged in 1× TBE buffer and then stained with ethidium bromide (10 mg ml^{-1}) solution for 20 min. The DNAs were visualized on a UV trans-illuminator and documented by using the Gel Documentation System of Alpha Innotech. Multi Imaging. The size of the amplified ISSR fragments was estimated by running 100 pb Ladder (Bio Rad) in the

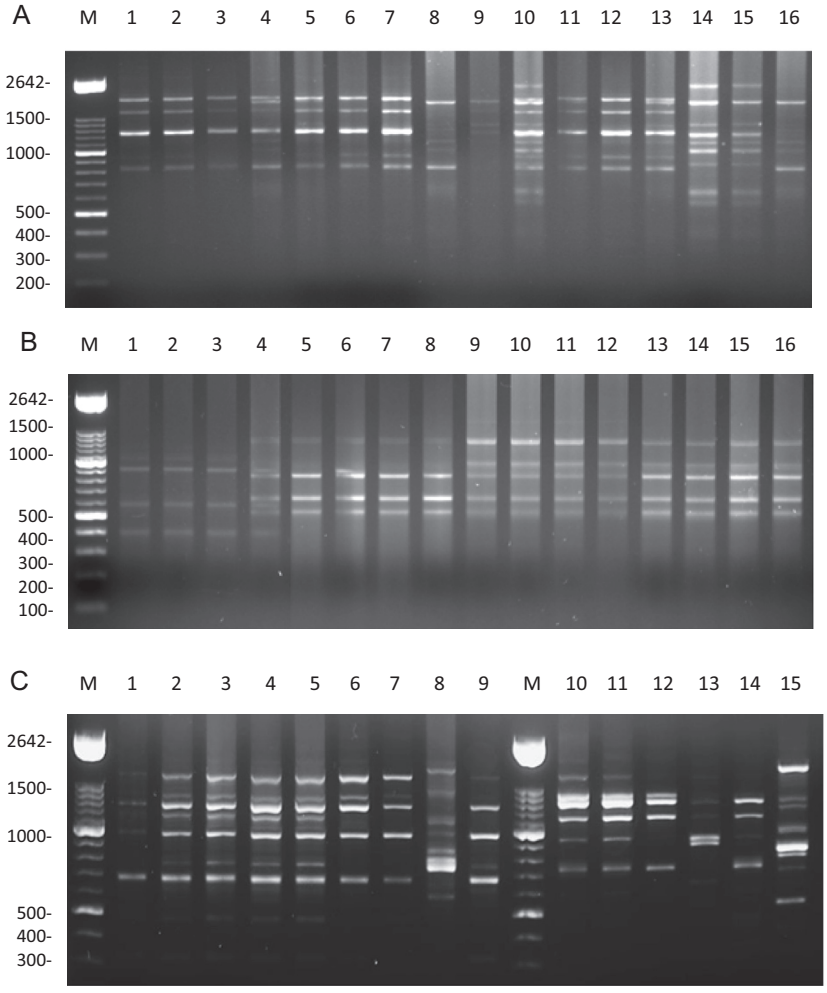

Figure 1 Photographs illustrating the ISSR fingerprinting revealed in 16 genotypes of Hassawi rice by primers 3 (A) and Primer 5 (B) and in 15 genotypes by primer 6 (C).

Table 2 Base sequence of the 11 ISSR primers which produced polymorphic fingerprinting in the 27 Hassawi rice genotypes and number of total alleles and number of amplified monomorphic and polymorphic bands as well as the percentage of polymorphism.

ISSR primer	Base sequence	No. of alleles	Polymorphic bands	Monomorphic bands	Percentage of polymorphism (%)
ISSR 1	(AG)8 T	17	17	0	100
ISSR 2	(AG)8 C	31	30	1	96.77
ISSR 3	(AG)8 G	12	9	3	75.0
ISSR 4	(GA)8 T	10	4	6	40.0
ISSR 5	(GA)8 C	11	9	2	81.81
ISSR 6	(GA)8 A	13	13	0	100.0
ISSR 7	(TC)8 C	5	2	3	40.0
ISSR 8	(AC)8 T	16	16	0	100
ISSR 9	(TG)8 A	14	14	0	100
ISSR 10	(CTC)6	17	17	0	100
ISSR 11	(AGG)5 CC	21	21	0	100
Total		167	152	15	90.02

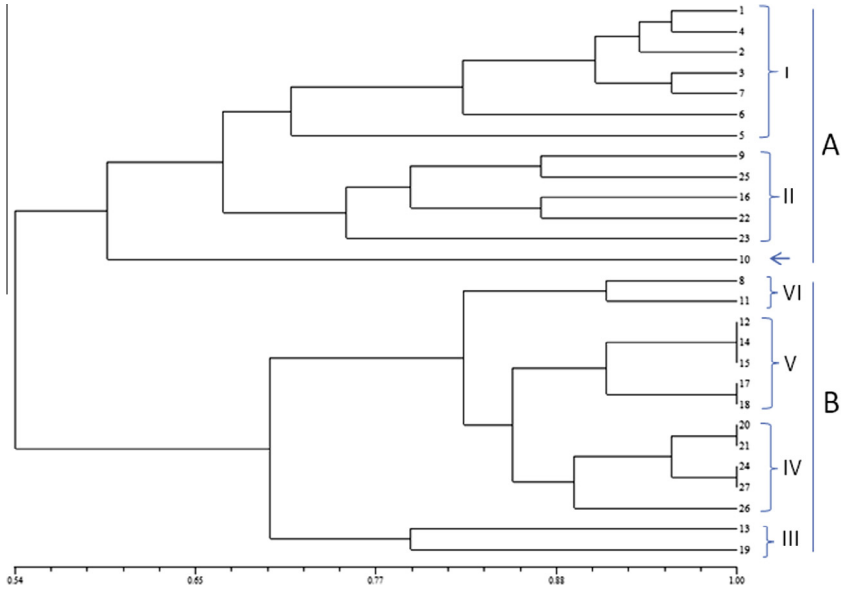

Figure 2 UPGMA tree illustrating the genetic diversity among 27 genotypes of Hassawi rice, based on ISSR markers and constructed using the NTSYS-pc software.

gel as standard size marker. To ensure the reproducibility and reliability of the ISSR markers we repeated the PCR reactions twice with each primer. The primers that showed weak or no patterns were discarded.

2.4. Data analysis

Since ISSR primers are dominant markers, amplified bands were scored 1 for presence or 0 for absence of bands. The similarity matrix was calculated among the examined 27 accessions based on Nei's genetic distance as implemented in the NTSYS-pc and the genetic distance among the landraces was expressed as a distance tree by using the NTSYS-pc software using un-weighted pair-group method with arithmetic averages (UPGMA) and simple matching coefficient (Rohlf, 2002). In addition, The Euclidean similarity coefficient among landraces was calculated according to Legendre and Legendre (1983) and genetic distance measures were performed using the Community Analysis Package Software Program (CAP) by Richard and Peter (2007). For the genetic distance tree construction, the agglomerative cluster analysis method was used according to Ward (1963).

3. Results and discussion

Eleven of 14 tested ISSR primers resulted in polymorphic ISSR profiles including six poly (GA) or (AG); di-nucleotide primers (Table 1). The number of bands produced by the used primers ranged between 5 for the primer 7 (TC)8 C and 31 for the primer 2 (AG)8 C. Primers numbered 1 (AG)8 T, 2 (AG)8 C, 8 (AC)8 T, 10 (CTC)6, and 11(AGG)5 CC (Table 1) produced higher number of total alleles compared to other primers. The total number of alleles produced by the eleven primers is 167 including 152 polymorphic markers and only 15 monomorphic markers. A 100% polymorphism was scored for six primers (Table 1). Meanwhile low polymorphism of 40% was scored for primer 7 and primer 4 (GA)8 T. The other three primers produced polymorphism ranging between 75%

Table 3 Similarity matrix, based on Nei's genetic distance, of 27 genotypes of Hassawi rice based on variation in ISSR markers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	1.000																										
2	0.916	1.000																									
3	0.916	0.916	1.000																								
4	0.958	0.958	0.958	1.000																							
5	0.750	0.666	0.666	0.708	1.000																						
6	0.875	0.791	0.791	0.833	0.791	1.000																					
7	0.875	0.875	0.958	0.916	0.708	0.833	1.000																				
8	0.750	0.750	0.750	0.791	0.583	0.791	0.791	1.000																			
9	0.833	0.750	0.750	0.791	0.833	0.791	0.708	0.583	1.000																		
10	0.541	0.541	0.541	0.500	0.708	0.500	0.500	0.291	0.708	1.000																	
11	0.750	0.750	0.750	0.791	0.500	0.708	0.708	0.916	0.666	0.375	1.000																
12	0.583	0.583	0.583	0.625	0.416	0.625	0.625	0.833	0.500	0.208	0.833	1.000															
13	0.541	0.625	0.625	0.583	0.375	0.583	0.666	0.708	0.458	0.333	0.708	0.875	1.000														
14	0.583	0.583	0.583	0.625	0.416	0.625	0.625	0.833	0.500	0.208	0.833	1.000	0.875	1.000													
15	0.583	0.583	0.583	0.625	0.416	0.625	0.625	0.833	0.500	0.208	0.833	1.000	0.875	1.000	1.000												
16	0.625	0.625	0.625	0.583	0.625	0.583	0.583	0.375	0.791	0.750	0.458	0.458	0.583	0.458	0.458	1.000											
17	0.666	0.666	0.666	0.708	0.416	0.625	0.625	0.833	0.583	0.291	0.916	0.916	0.791	0.916	0.916	0.541	1.000										
18	0.666	0.666	0.666	0.708	0.416	0.625	0.625	0.833	0.583	0.291	0.916	0.916	0.791	0.916	0.916	0.541	0.985	1.000									
19	0.583	0.666	0.666	0.625	0.416	0.625	0.708	0.666	0.416	0.375	0.583	0.666	0.791	0.666	0.666	0.625	0.666	0.666	1.000								
20	0.625	0.625	0.625	0.666	0.375	0.583	0.583	0.791	0.541	0.250	0.875	0.875	0.750	0.875	0.875	0.416	0.875	0.875	0.625	1.000							
21	0.625	0.625	0.625	0.666	0.375	0.583	0.583	0.791	0.541	0.250	0.875	0.875	0.750	0.875	0.875	0.416	0.875	0.875	0.625	0.981	1.000						
22	0.666	0.666	0.666	0.625	0.666	0.625	0.625	0.416	0.833	0.708	0.500	0.500	0.625	0.500	0.500	0.875	0.500	0.500	0.500	0.541	0.541	1.000					
23	0.708	0.625	0.625	0.666	0.625	0.666	0.583	0.458	0.791	0.500	0.541	0.541	0.500	0.541	0.541	0.666	0.541	0.541	0.458	0.666	0.666	0.791	1.000				
24	0.583	0.583	0.583	0.625	0.333	0.541	0.541	0.750	0.500	0.208	0.833	0.833	0.708	0.833	0.833	0.375	0.833	0.833	0.583	0.958	0.958	0.500	0.625	1.000			
25	0.708	0.625	0.708	0.666	0.708	0.666	0.666	0.458	0.875	0.666	0.541	0.375	0.416	0.375	0.375	0.750	0.458	0.458	0.375	0.416	0.416	0.791	0.750	0.375	1.000		
26	0.666	0.666	0.666	0.708	0.416	0.625	0.625	0.833	0.583	0.291	0.916	0.833	0.708	0.833	0.833	0.458	0.916	0.916	0.583	0.875	0.875	0.500	0.541	0.916	0.458	1.000	
27	0.583	0.583	0.583	0.625	0.333	0.541	0.541	0.750	0.500	0.208	0.833	0.833	0.708	0.833	0.833	0.375	0.833	0.833	0.583	0.958	0.958	0.500	0.625	0.981	0.375	0.916	1.000

for primer 3 (AG)8 G and 96.77% for primer 2 (AG)8 C. The total percentage of polymorphic markers for all primers in the examined 27 accessions is 90.02%, which indicated high level of genetic variation among the examined land races of Hassawi rice.

The number of alleles per primer varied between 5 for primer 7 and 31 for primer 2, with a mean of 15.18. Examples of photographs illustrating the ISSR fingerprinting of some landraces by selected primers are shown in Fig. 1A–C. These include ISSR fingerprinting revealed in 16 landraces of Hassawi rice by primers 3 (AG)8 G (A) and Primer 5 (GA)8 C (B) and in 15 landraces by primer 6 (GA)8 A (C). The approximate size of the largest fragment produced was 2.5 kbp and the smallest easily recognizable fragments produced was approximately 0.26 kbp. The report by Blair et al. (1999) that dinucleotide primers yield highly informative patterns compared to tri- and tetra-nucleotide primers in rice indicating that the dinucleotide repetitive sequence primers are more amenable to ISSR analysis than the tri- and tetra-nucleotide primers is not supported by our findings in the Hassawi rice as the two tri-nucleotide primers numbered 10 and 11 produced 17 and 21 polymorphic alleles respectively.

The pair-wise genetic similarity estimates, based on Nei's similarity coefficient, of the 27 landraces used in this study are given in Table 2. The similarity coefficient ranged from 1.000 to 0.208. Maximum similarity was observed between landraces of Hassawi rice numbered 12, 14 and 15 (1.000). The second highest similarity was between landraces numbered 17 and 18 (0.985) and the third highest similarity (0.981) was between the two landraces numbered 24 and 27 and the two landraces 20 and 21 respectively.

The genetic distance tree made using cluster analysis by the UPGMA method in the NTSYS-pc showed two main groups, A and B (Fig. 2). In group A, the landrace numbered 10 was clearly differentiated from two clusters (I & II). Cluster I comprised of seven landraces (numbered 1, 2, 3, 4, 5, 6 & 7) with a 0.958–0.708 Nei's similarity range. In this cluster two pairs of landraces i.e. 1 & 4, and 3 & 7 showed close similarity value (0.958). Cluster II consisted of five landraces (numbered 9, 16, 22, 23 and 25) with a range of 0.875–0.666 coefficient values in the similarity matrix. Two pairs of landraces in this cluster i.e. (9 & 25) and (16 & 22) have the same similarity value of 0.875. For KACST IDs corresponding to the serial numbers mentioned above see Table 1.

Group B consisted of three clusters; cluster III consisted of only two landraces (numbered 9 & 13) with a Nei similarity coefficient of 0.916. Cluster IV consisted of five landraces (numbered 12, 14, 15, 17 & 18) that are the most closely related landraces among all the 27 accessions studied, with the highest value in the similarity matrix among 12, 14 and 15 (1.000) and second highest among 17 and 18 (0.985). Cluster V is also comprised of five closely related landraces (numbered 20, 21, 24, 26 & with high similarity value between them. Meanwhile the two landraces (numbered 8 & 11) with Nei's similarity coefficient of 0.791 represent cluster VI of the examined 27 landraces (for KACST IDs, see Table 1).

The pair-wise genetic similarity estimated as Euclidean coefficient among the 27 landraces used in this study is given in Table 3. The similarity coefficient ranged from 9.41 and 4.06. High similarity was observed between some landraces pairs such as 1 & 4 (9.20), 3 & 7 (9.24), 12 & 14 (9.27), 20 & 21 (9.32) and 24 & 27 (9.41) and lower similarity coefficients were particularly observed between landrace 10 and several other landraces such as 8, 14 and 15 (Table 3); (for KACST IDs, see Table 1).

The distance tree illustrating the genetic distance using cluster analysis made by the Ward method in the CAP software also divided the 27 rice landraces into two main groups, A and B at a distance of 159 on the Ward distance scale (Fig. 3). The groups in this tree are similar to those produced in the UPGMA tree (Fig. 2). In group A, the landrace numbered 10 was clearly differentiated from two clusters (I and II) at a distance of about 90. Cluster I and cluster II comprised the same landraces that were also clustered together in the UPGMA tree made using the NTSYS-pc. Cluster I comprised of the seven landraces (numbered 1, 2, 3, 4, 5, 6 and 7) with

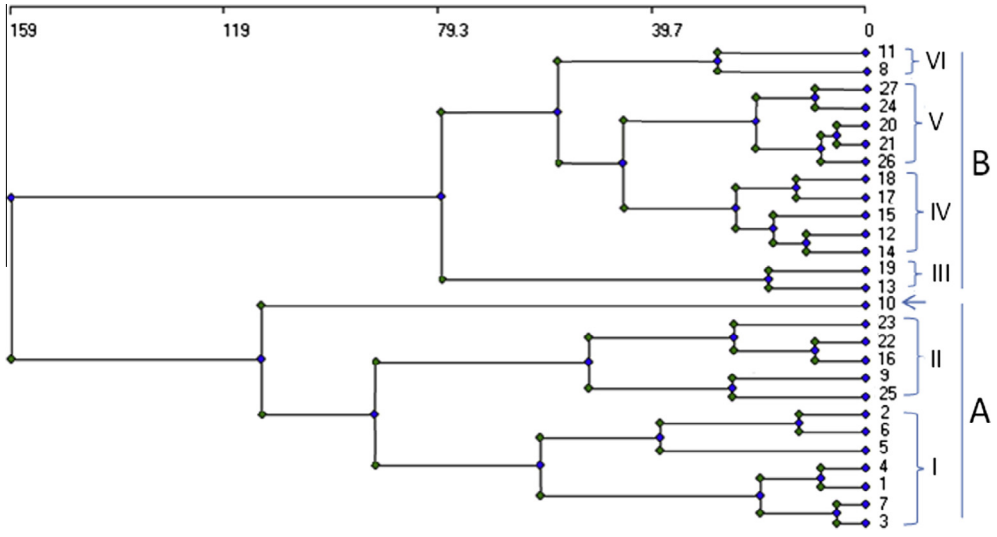

Figure 3 Ward tree illustrating the genetic diversity among 27 genotypes of Hassawi rice based on ISSR markers and constructed using the CAP-pc software.

Table 4 Similarity matrix, based on Euclidean distance of 27 genotypes of Hassawi rice based on variation in ISSR markers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	1																										
2	8.80	1																									
3	8.36	8.16	1																								
4	9.20	8.47	8.00	1																							
5	8.75	6.47	8.69	7.69	1																						
6	8.29	8.58	8.12	7.80	7.32	1																					
7	8.57	8.47	9.24	7.69	6.24	6.61	1																				
8	7.94	7.87	8.12	7.87	7.75	8.55	7.62	1																			
9	8.54	8.60	8.94	8.83	8.49	8.43	8.60	8.10	1																		
10	8.43	8.06	8.06	8.17	8.17	8.78	8.83	4.93	6.00	1																	
11	8.25	7.66	8.66	8.89	8.78	8.49	8.54	8.68	6.40	5.19	1																
12	8.49	7.89	7.11	9.00	8.78	8.72	8.78	8.42	6.86	5.40	7.69	1															
13	8.49	7.19	8.31	8.31	8.54	8.49	8.19	8.55	7.14	5.86	8.66	6.16	1														
14	8.83	7.66	7.89	8.66	8.78	8.83	8.66	8.06	7.55	4.06	8.35	9.27	7.48	1													
15	8.29	7.72	7.94	8.49	8.49	8.66	8.72	7.75	7.62	4.37	7.28	8.14	5.92	8.12	1												
16	8.49	8.16	7.66	8.43	8.54	8.60	8.66	7.68	6.42	5.81	7.07	8.35	6.33	6.83	7.20	1											
17	8.06	7.12	7.25	7.87	8.00	8.19	8.37	7.62	7.62	5.37	7.81	828	8.56	5.75	6.10	6.36	1										
18	8.43	7.83	7.94	8.60	8.60	8.43	8.94	7.75	7.48	7.62	7.81	8.94	7.68	8.56	8.00	5.06	8.00	1									
19	8.60	7.78	7.11	9.00	8.66	8.72	6.11	7.55	7.55	7.92	8.12	6.12	9.11	8.37	8.06	8.37	9.11	612	1								
20	7.12	7.94	7.19	8.31	8.31	8.00	8.43	7.68	8.06	5.54	8.00	8.00	8.12	8.94	8.78	8.94	8.22	7.00	6.33	1							
21	7.94	7.62	7.87	7.75	7.87	7.68	8.00	7.62	8.12	8.72	8.19	7.19	8.31	8.89	8.6	8.66	6.06	7.62	6.86	9.32	1						
22	8.81	7.35	7.48	7.75	7.35	7.00	7.62	7.48	7.48	7.12	7.68	7.31	7.68	8.19	7.75	8.94	8.00	7.75	7.28	6.08	6.50	1					
23	8.00	7.55	7.55	7.42	7.28	6.93	7.42	7.55	7.94	8.43	8.12	8.72	7.75	8.00	7.55	8.35	7.81	8.54	6.37	8.62	7.10	9.00	1				
24	7.06	7.62	7.75	7.75	7.21	7.28	7.35	8.62	7.87	4.25	7.94	7.94	7.00	7.42	7.21	6.86	7.78	6.72	6.78	6.11	8.80	7.48	5.75	1			
25	8.00	7.94	7.94	8.06	7.81	8.00	7.68	8.06	9.06	4.94	7.87	8.00	7.62	7.62	7.94	8.62	7.55	6.11	6.49	6.90	10.0	6.22	8.37	6.40	1		
26	7.75	7.81	7.81	7.68	7.94	8.00	7.81	8.06	8.43	5.31	7.87	8.12	8.00	7.62	7.81	7.75	7.81	7.43	8.06	8.89	9.30	6.43	6.17	8.61	6.00	1	
27	7.55	7.48	7.21	7.21	7.35	7.28	7.48	9.00	8.12	4.60	8.19	8.31	8.31	7.81	8.12	8.06	6.10	7.60	8.11	8.11	8.70	8.72	6.19	9.41	6.68	6.56	1

close resemblance between landraces 1 & 4, and 3 & 7 and 2 & 5. Cluster II also consisted of the five landraces (numbered 9, 16, 22, 23 and 25). The landraces 9 & 25 clustered together and the landraces 16, 22 & 23 are clustered together as close landraces (for KACST IDs, see Table 1).

Group B consisted of four clusters; cluster III consisted of the two landraces 13 and 19 at a relatively high distance from cluster IV and cluster V. Cluster IV composed of the five landraces 12, 14, 15, 17 & 18 with close similarity between the two landraces 9 & 25 and the three landraces 16, 22 & 23. Cluster V comprised of five closely related landraces, two numbered (24 & 27) were clustered together and the landraces numbered 20, 21 & 26 were grouped together at small distance. The two landraces numbered 8 & 11 were differentiated from other landraces as cluster VI. It is to be noted that the landraces grouped together in the same group are mostly accessions that were collected from sites that are geographically closer compared to accessions delimited in different groups.

The foregoing results indicate that ISSR markers are very informative and cost-effective in determining genetic diversity among diverse accessions of Hassawi rice germplasm growing in Saudi Arabia. This evidence is congruent with previous reports on the usefulness of ISSR markers if differentiating rice genotypes (Joshi et al., 2000; Davierwala et al., 2000; Sarla et al., 2003, 2005). Reddy et al. also claimed a link between GA repeats and tolerance of rice varieties to different abiotic stresses as evidenced by grouping of the stress-tolerant landraces when (GA)8 YG was used. The examined materials were gathered from a landrace of rice that is known to be drought and salinity tolerance (Al-Mssallem and Al-Mssallem, 1997) but their clustering based on tolerance based on ISSR amplification with certain primers was not tested; this may be one of the future objectives based on the clustering of the examined 27 landraces by the used primers. However the grouping of accessions growing in close geographic proximity is congruent with the findings of Sarla et al. (2003) that ISSR markers based on AG and GA repeats delineated geographically diverse *Oryza nivara* accessions. (see Table 4).

These data reported here also help in genetic authentication of the rice germplasm in the gene bank held by King Abdel-Aziz City for Science and Technology. The results encourage more comprehensive screening of more landraces using more ISSR primers and as well as additional molecular as well as phenotypic markers since insights from the phenotypic traits of rice are likely to be validated with subsequent molecular analysis (Ray et al., 2013) for better assessment of genetic diversity among the rice land races in Saudi Arabia. The information is given in this report is however a step toward establishing the breeding stock for future improvement of rice production in the kingdom, which is one of the most consuming rice countries in the world. Such objectives may help in selecting potential landraces for breeding new lines.

In conclusion, the ISSR analysis divided 27 landraces of Hassawi rice into two major genetically diverse groups, A and B and distinguished certain genotypes in the region. The ISSR markers as reported here thus adds useful information for estimating the genetic diversity among rice germplasm in Al-Ahsa region and for the use of rice landraces in Saudi Arabia in future breeding of new cultivars of rice that may be more tolerant to abiotic stresses, more productive and have better nutritive value compared to the current landraces.

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