

Genetic Diversity of Some Saudi Barley (*Hordeum Vulgare* L.) Landraces Based on Two Types of Molecular Markers

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Abstract: Problem statement: Very little is known about the genetic diversity and morphological variability present in barley landrace in KSA, a country experiencing loss of biodiversity because of replacement of landraces with modern landraces. **Approach:** The molecular markers RAPD and ISSR were used as an efficient tools to estimate the *intra*- and *inter*-cultivar polymorphism among six barley KSA landraces collected from different geographical regions in order to assess the genetic relationships and develop cultivar-specific molecular fingerprints. The long term objective was to use these fingerprints to identify molecular markers that co-segregate and could be used in isolating gene(s) which controlling some important traits, thereafter could be used in breeding programs (marker assisted selection). **Results:** Out of 20 and 10 primers of RAPD and ISSR, respectively, a clear and reproducible band profile of 13 RAPD primers and 7 ISSR primers were obtained. In RAPD analyses, 61 out of 111 bands (54.6%) were polymorphic. The number of alleles ranged from 5-15 per primer, with an average of 8.54 per primer. In ISSR analyses, a total of 53 alleles were detected, among which 16 alleles (30.2%) were polymorphic. The number of alleles per primer ranged from 5-10 with an average of 7.57 alleles per ISSR primer. The mean Polymorphism Information Content (PIC) values were 0.45 and 0.37 for RAPD and ISSR markers, respectively. **Conclusion:** ISSR is better than RAPD to detect genetic diversity among the barley landraces. The RAPD's and ISSR's have confirmed each other and the ISSR results are more realistic comparing to RAPD results regarding to the geographical distribution of the six barley landraces. The outcome of this investigation can help strengthen the exiting pool of information on barley that may help assess national barley programs in KSA.

Key words: Barley, Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), Polymorphism Information Content (PIC), Kingdom of Saudi Arabia (KSA)

INTRODUCTION

Barley was first cultivated 10,500 years ago in the Fertile Crescent and is one of the founder crops of Eurasian agriculture (Saisho and Purugganan, 2007). In some regions of the world, especially the sub-regions, barley landraces still cultivated. Barley landraces are considered as the evolutionary link between wild barley (*Hordeum spontaneum* K. Koch) and modern barley landraces. Barley landraces are genetically heterogeneous populations comprising inbreeding lines and hybrid segregates generated by a low level of random out crossing in each generation (Shewry, 1992). Moreover, having evolved across thousands of years in a multitude of environments and local farming systems,

these landraces have developed abundant patterns of variation and would represent a largely untapped reservoir of useful genes for adaptation to biotic and abiotic stresses (Brush, 1995). Assessment of the extent of genetic variability within barley, including the wild relatives, is fundamental for barley breeding and the conservation of genetic resources and is particularly useful as a general guide in the choice of parents for breeding hybrids (Hou *et al.*, 2005).

In the Kingdom of Saudi Arabia (KSA), less than 2 percent of the land is used for cultivation. Crops are grown mainly in the southwest of the kingdom, where there is rainfall sufficient for farming, or in areas where oases provide enough ground water for irrigation. Landraces of important crops such as barley and wheat

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have persisted in situ because of the nature of the farming systems. By growing a mixture of diversified local materials, farmers were always able to select varieties adapted to local environmental conditions. Since the 1990s, many modern landraces of barley were introduced to the kingdom to replace the local landraces resulted in the gradually decrease of these variable source of biodiversity. However, barley landraces can still be found in isolated and marginal areas, such as mountains and oases where traditional landraces are still grown. Very little is known about the genetic diversity present in these barley landrace.

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Russell *et al.*, 1997). It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay (Davila *et al.*, 1999a; 1999b). The advent of the Polymerase Chain Reaction (PCR) favored the development of different molecular techniques such as Random Amplified of Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR or microsatellite), Sequence Tagged Sites (STS), Random Amplified Microsatellite Polymorphism (RAMP) and Inter-Simple Sequence Repeat (ISSR) and so on (Nagaoka and Ogihara, 1997). These molecular markers had been used in barley for detecting genetic diversity, genotype identification, genetic mapping (Tanyolac, 2003). Of these techniques, RAPD has several advantages, such as simplicity of use, low cost and the use of small amount of plant material. RAPDs were proved to be useful as genetic markers in the case of self-pollinating species with a relatively low level of intra specific polymorphism, such as hexaploid wheat (Joshi and Nguyen, 1993) and cultivated barley (Tinker *et al.*, 1993). ISSR markers, which involve PCR amplifications of DNA using a primer composed of a microsatellite sequence anchored at 3' or 5' end by 2-4 arbitrary, could be used to assess genetic diversity (Qian *et al.*, 2001). ISSRs have been used for cultivar identification in maize (Pejic *et al.*, 1998), potatoes (Prevost and Wilkinson, 1999), wheat (Nagaoka and Ogihara, 1997), bean (Metais *et al.*, 2000), Diplotaxis (Martín and Sanchez-Yelamo, 2000) and barley (Hou *et al.*, 2005).

The major objectives of this work were focusing on estimating of polymorphism among six barley KSA landraces, Hail; Qassem; Taif; Gizan; Bahah and Asser, using RAPD and ISSR molecular markers, as efficient tools in order to assess the genetic relationships and develop genotype-specific molecular fingerprints to be used in barley breeding programs.

MATERIALS AND METHODS

Materials: Six barley landraces samples were collected from different locations of KSA and named according to the collection area as Hail; Qassem; Taif; Gizan; Bahah and Asser.

Methods: Isolation and purification of genomic DNA from the different barley landraces.

Fresh young healthy barley leaves were collected from the studied barley landraces and grounded to powder with liquid N₂ using a mortar and pestle. Genomic DNA was isolated from leaf samples using the procedure described by the plant isolation kit (Jena Bioscience, Germany).

RAPD analysis:

PCR reaction and condition: A total of 20 random primers were used to detect the polymorphism. The sequence of the 13 primers that produce a clear scorable and reproducible banding pattern is shown in (Table 1). The amplification performed in a 25 µl reaction volume containing about 3 µl (10 ng µL⁻¹) genomic DNA, 3 µL primer (Operon Technologies Inc.) and 12.5 µL master mix (Promega) and 6.5 µL of PCR water. The PCR temperature profile was applied through Bio-Rad C1000 thermal cycler (Germany) that was programmed with an initial step of 5 min at 94°C; the amplification reaction was carried out using 40 cycles of 60 s at 94°C, an annealing step of 1 min at 37°C and an elongation step of 1min at 72°C; and finally a 7 min extension at 72°C. The amplification products were resolved by electrophoresis in a 1, 5% agarose gel containing ethidium bromide (0.5 µg mL⁻¹) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000).

ISSR analysis: Ten ISSR primers were tested using a specific and optimal annealing temperature for each one (Table 2). PCR reactions were performed in a volume of 20 µl Bio-Rad C1000 thermal cycler (Germany). The reaction mixture contained 0.6 µL of each primer, 100 µM of each deoxynucleotide, 0.5 units of Go Taq polymerase (Promega), 10×Taq buffer containing 2.5 mM MgCl₂ and 10 ng of template DNA. Amplification reaction was 94°C/5 min, followed by 30 cycles of 94°C/1 min, 4-60°C (specific for each primer)/1 min and 72°C/2 min and ending with an extension step of 7°C / 7 min. PCR products were analyzed using agarose (2% w/v) electrophoresis gels stained with ethidium bromide and only bands with high intensity and well separated were selected.

Table 1: List of random primers that have been used for RAPD analysis with their sequence, polymorphism % and Polymorphic information content value (PIC) for each marker

Primer No	Primer name	Primer sequence	Number of total bands	Amplified fragments (bp)	Polymorphism (%)	PIC
1	OP-A06	GGTCCCTGAC	5.0	200-1700	20.0	0.32
2	OP-A07	GAAACGGGTG	9.0	500-2000	25.0	0.38
3	OP-A08	GTGAGGTAGG	9.0	600-1500	33.3	0.45
4	OP-A10	GTGATCTCC	7.0	350-1600	43.0	0.50
5	OP-B01	TGATCCCTGG	8.0	400-3000	100.0	0.90
6	OP-B02	GGACTGGAGT	6.0	500-2500	50.0	0.50
7	OP-B03	TGCGCCTTC	5.0	400-1600	40.0	0.48
8	OP-B04	TGCGCCCTTC	6.0	700-2000	17.0	0.28
9	OP-B05	CTGCTGGGAC	11.0	400-2000	64.0	0.46
10	OP-B10	TTCGAGCCAG	11.0	200-1500	42.0	0.49
11	OP-C01	GATGAGCCAG	6.0	100-2000	50.0	0.50
12	OP-C02	GATGACCGCC	7.0	600-1500	70.0	0.42
13	OP-C03	TGGACCGGTG	13.0	300-1700	93.0	0.13
Average			7.8		49.8	0.45

Table 2: List of Primers that have been used for ISSR analysis with their sequence, Amplified Fragments (bp), Number of total bands, Polymorphism % and PIC value for each marker

Primer Name	Primer sequence	Number of total bands	Amplified fragments (bp)	Polymorphism (%)	PIC
1-UBC80	ATATATATATATATATT	-	-	-	-
2-UBC807	AGAGAGAGAGAGAGAGT	5.0	500-2000	-	-
3-UBC828	TGTGTGGTGGATGTGGAT	6.0	400-1700	33.30	0.41
4-UBC878	CATGGTGTGGATGGAT	6.0	400-1000	33.30	0.45
5-UBC899	CATGGTGGTGCATTGTTC	-	-	-	-
6-UBC822	TCTCTCTCTCTCTCA	8.0	500-1800	25	0.47
7-UBC814	CTCTCTCTCTCTCTA	-	-	-	-
8-UBC835	AGAGAGAGAAGAGAGAYT	10.0	800-1000	30.00	0.42
9-UBC840	GAGAGAGAGAGAGAYT	10.0	100-2000	30.00	0.42
10-UBC847	CACACACACACACARC	6.0	300-500	33.30	0.45
Average		7.4		25.80	0.37

Data scoring and statistical analysis: To ensure the absence of artifacts, bands were carefully selected from replicated amplifications (three times). Amplified bands designated by their primer code and their size in base pairs. Data recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. Only intense and reproducible bands appearing on the gel were scored. Band scoring was analyzed using Gene Tools-gel analysis software of SPSS ver. 16. The Polymorphic Information Content value (PIC) refers to the value of a marker for detecting polymorphism within a population and depends on the number of detectable alleles and the distribution of their frequency. PIC was calculated using the equation:

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

where, PIC_i is the polymorphic information content of a marker i; P_{ij} is the frequency of the jth pattern for marker i and the summation extends over n patterns (Anderson *et al.*, 1993).

RESULTS

RAPD-PCR fingerprinting of barley landraces: The use of RAPD's molecular markers was aiming to show fast and reliable discrimination of any variations. The DNA of the six landraces of KSA barley was used as templates for 20 RAPD primers.

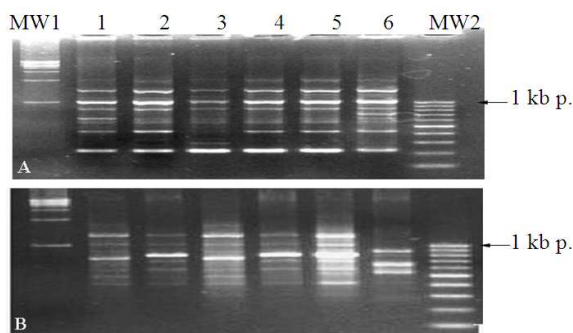


Fig. 1: RAPD fingerprinting of 6 KSA barley landraces (*Hordium vulgare* L.). MW1: is 1 kb. ladder and MW2 is 100 bp. ladder. (1) Taif; (2) Hail; (3) Qassem; (4) Asser; (5) Bahah; (6) Gizan. (A) Primer OP-B10; and (B) Primer OP-C08

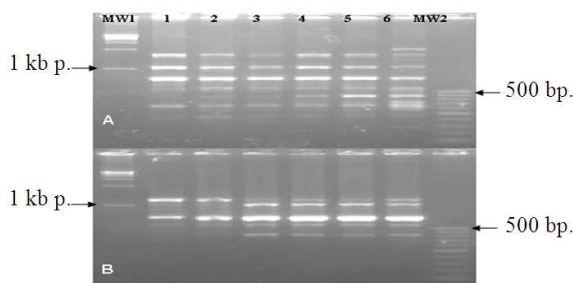


Fig. 2: ISSR fingerprinting of 6 KSA barley landraces (*Hordium vulgare* L.). MW1: is 1 kb. ladder and MW2 is 50 bp. ladders. (1) Taif; (2) Hail; (3) Qassem; (4) Asser; (5) Bahah; (6) Gizan. (A) Primer UBC840 and (B) Primer UBC847

Only 13 primers produced a clear, scoreable banding pattern. Number of bands obtained with each primer, polymorphism % and PIC value of each primer were illustrated in Table 1.

Total of 102 bands were obtained with the average of 7.8 bands for each primer. The highest band number was produced with primer OP-C08 (15 bands) while the lowest was produced with primers OP-A06 and OP-B03 (5 bands). The RAPD banding obtained with primers OP-B10 and OP-C05 are illustrated in Fig. 1. The size of the amplified fragments was ranged between 100 and 3000 bp length (Table 1). Primer OP-B01 showed the highest polymorphism (100%), while primer OP-B04 showed the lowest with only 17% (Table 1). Remarkably, primer (OP-B05) has showed specific product for Qassem and Bahah landraces, between 300 and 400 bp. length. While primers OP-B10 has showed a specific product for Qassem landrace between 400 and 500 bp. length (Fig. 1). The primer OP-C08 has showed a specific product for Gizan cultivar, with 800 bp. length.

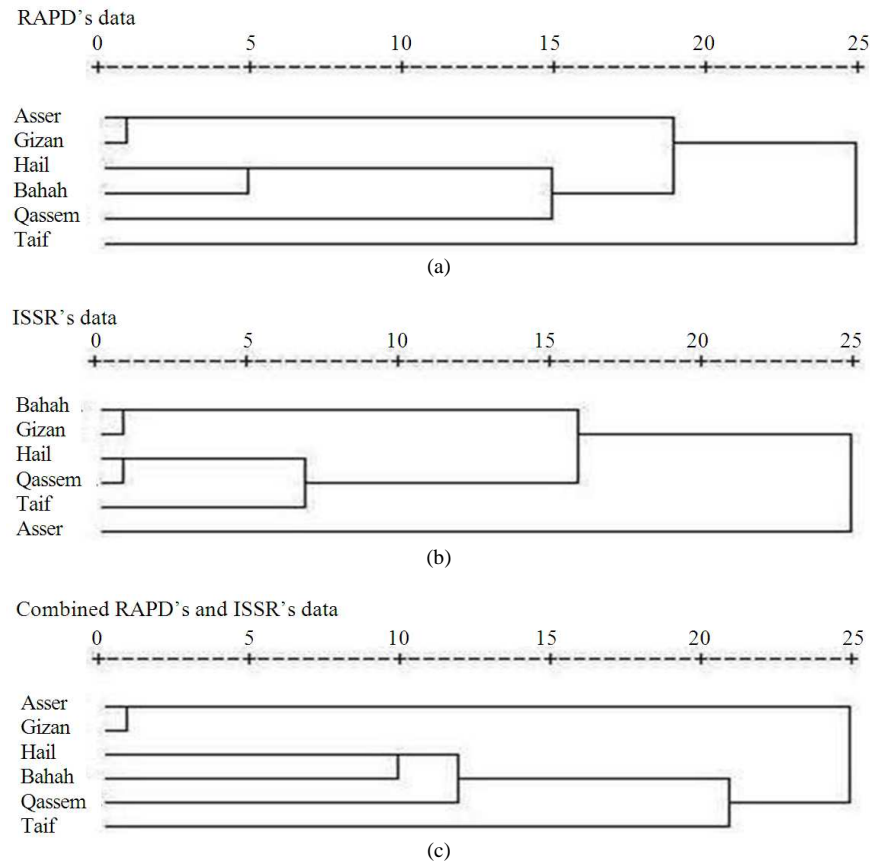


Fig. 3: Phylogenetic analysis among the six barley landraces using RAPD and ISSR. (A) the analysis of RAPD's data; (B) the analysis of ISSR's data and (C) the combined RAPD's and ISSR's data

ISSR-PCR fingerprinting of barley landraces: In this study, we have used the DNA of six KSA barley landraces, as templates for 10 ISSR primers. However, only seven primers showed clear and scorable bands. The ISSR-PCR reaction of primer (UBC807) has recorded 5 fragments that with the molecular size ranged from 500-2000 bp. All five bands were observed in all landraces and exhibited 100% monomorphism (data not shown). The other six ISSR primers exhibited a polymorphic banding pattern.

The total of 51 bands were obtained with the average of 7.4 bands for each primer. The highest band number was produced with primers UBC835 and UBC840 (10 bands) while the lowest was produced with primers UBC807 (5 bands). The size of the amplified fragments was ranged between 300 and 2000 bp length (Table 2). The three primers UBC828, UBC878 and UBC847 showed polymorphism % of (33.3), while primer UBC822 showed the lowest with only 25% (Table 2). The ISSR banding pattern obtained with primers UBC840 and UBC847 are depicted in Fig. 2. A specific band for Gizan landrace,

with around 2000 bp. Length was produced by the primer UBC840 has showed a specific (Fig. 2-A)

Genetic relationships as revealed by RAPD and ISSR markers: The RAPD's product showed a genetic similarity ranged from 59.6-84.4% (Table 3). The highest genetic similarity revealed by the RAPD's analysis was 84.4% between landraces Asser and Gizan and 80.9% between landraces Hail and Bahah. On the other hand, the lowest genetic similarity was 59.6% between landraces Qassem and Taif.

The ISSRs data revealed a genetic similarity ranged from 76-93.6% (Table 3). The highest genetic similarity revealed by the ISSR analysis was 93.6% between Bahah and Gizan landraces and 93.6% between landraces Hail and Qaseem. On the other hand, the lowest genetic similarity was 76% between Asser and Taif landraces. The ISSR data was very close to the geographical distribution of the six landraces.

The RAPD's and ISSR's combined data were close to RAPD data and showed genetic similarity ranged between 68.2 and 84.6% (Table 3).

Table 3: Genetic dissimilarity (GS) matrices computed according to Dice coefficient from RAPD and ISSR markers of six KSA barley landraces. (Taif; Hail; Qassem; Asser; Bahah; and Gizan). (A): is the analysis of RAPD's data; (B): is the analysis of ISSR's data and (C): is the combined RAPD's and ISSR's data

Case	Taif	Hail	Qassem	Asser	Bahah	Gizan
A RAPD's data						
Taif						
Hail	0.745					
Qassem	0.596	0.710				
Asswer	0.644	0.750	0.692			
Bahah	0.695	0.809	0.774	0.701		
Gizan	0.636	0.727	0.685	0.844	0.713	
B ISSR's data						
Taif						
Hail	0.898					
Qassem	0.917	0.936				
Asswer	0.760	0.776	0.830			
Bahah	0.843	0.860	0.917	0.872		
Gizan	0.820	0.837	0.894	0.848	0.936	
C Combined RAPD's and ISSR's data						
Taif						
Hail	0.796					
Qassem	0.704	0.786				
Asswer	0.682	0.758	0.739			
Bahah	0.747	0.826	0.826	0.757		
Gizan	0.698	0.764	0.657	0.846	0.787	

The highest genetic similarity was 84.6% between Asser and Gizan, while the lowest genetic similarity was 68.2% between Taif and Asser landraces.

Cluster analysis as revealed by RAPD and ISSR markers: Although barley landraces, Hail, Qassem and Taif were collected from the same geographical region of KSA and Bahah, Asser and Gizan were collected from another geographical region, RAPD's product in Fig. 3 showed that in a dendrogram the six barley landraces cluster comprised two major sub clusters with 19% dissimilarity. One subcluster includes (Hail, Bahah and Qassem), with genetic similarity with similarity starts from 71% until 80.9%. The other subcluster includes two barley landraces (Gizan and Asser), with similarity of 84.4%. While the sixth cultivar, Taif, is falling outside of both of two subclusters (Fig. 3). This means that the environmental and biotic conditions are very powerful in causing genetic variations among barley landraces. Moreover, the classification of these varieties might be based on morphological traits which is not so powerful comparing with the molecular markers.

ISSR results in (Fig. 3) showed that in a dendrogram the six landraces cluster comprised two subclusters with almost 16% dissimilarity. One subcluster includes (Hail, Taif and Qassem), with genetic similarity starts from 89.8% until 93.6%. The other subcluster includes two barley landraces (Gizan

and Bahah), with similarity of 93.6%. While the sixth landrace Asser is falling outside of both of two subclusters. The ISSR results confirmed the geographical distribution, when the Hail, Taif and Qassem fell together in one group, also the other three landraces, Gizan, Asser and Taif recorded high similarity, but some speciation pressures could cause the genetic differences among the three landraces. Although the ISSR data is logically close to the nature distribution of the studied landraces, the combined RAPD and ISSR results was relatively close to the RAPD data (Fig. 3).

DISCUSSION

It is obvious that the varieties landraces in different ecological conditions of KSA have attained different genetic profiles during the time. Most important criteria for the selection of marker are: Amount of available information, simple process or work, less expenses, high rate of doing work. In attention to the fact that RAPD marker don't need the use of radioactive elements and having sample sequence in plant genome such as possibility of the use of general primers those can be applied in every species of plant therefore RAPD marker is the suitable marker for the study of genetic variations and relativity rate and also it has the ability to assess the reservoirs of inheritance of the plant species.

Due to its worldwide distribution, the assessment of the genetic diversity among barley germplasm from different countries was performed (Fernandez *et al.*, 2002; Feng *et al.*, 2003). In fact, it has been found that the average genetic diversity based on RAPD analysis of 18 barley accessions from Netherlands, France, Great Britain, Germany and Italy was 0.521 (Russell *et al.*, 1997). In other conducted work on 30 barley American landraces (Pillen *et al.*, 2000), the average genetic diversity was 0.682. Whereas, the genetic diversity between tea populations (Kaundun and Park, 2002) was 0.18.

In the present study, the average genetic dissimilarity of barley landraces was 0.42. These results suggest that the selected barley landraces have narrow genetic diversity, despite the high PIC value of primers used. Correlation between RAPD markers and the geographic origin is low: In deed Bahah landrace from the south of KSA was closely related to Hail and Qassem landraces which is from north regions of KSA. The landraces Taif and Qassem, collected from the same region, showed an important Genetic Diversity (GD) of 0.59%. However, Hail and Qassem belonging to the same bioclimatic stage showed a low GD. This study corroborate with those found in Korean tea

populations (Kaundun and Park, 2002). This relatively poor relationship observed between RAPD markers and the geographic origin of KSA barley landraces in this study may be explained by the neutrality of molecular markers compared with adaptive traits under harsh conditions. This study revealed the efficacy of RAPD markers in detecting the polymorphism among barley landraces and establishing relationship among them. It demonstrated the potential efficiency of molecular markers in landraces classification and indicated the feasibility of a comprehensive effort to determine the relationships among barley landraces using molecular markers. Further collection, evaluation and utilization of local germplasm, is clearly a high priority in barley improvement.

In this study, it was obvious that the dendrogram based on RAPD markers was not in accord with the dendrogram based on ISSR markers. The dendrogram generated by the ISSR matrix agrees better with the geographic origins of the genotypes than the dendrogram generated by the RAPD results. For example, three landraces Hail, Qassem and Taif (originating from north KSA), while the other three landraces, Bahah, Gizan and Asser (from south KSA) were closely clustered in the dendrogram generated by the ISSR matrix and agrees better with the geographical distribution, comparing with the RAPD dendrogram (Fig. 1a and b). Moreover, (GS) matrices computed according to Dice coefficient from ISSR markers of the six KSA barley landraces were high between those landraces that collected from the same geographical region of KSA (Table 3).

CONCLUSION

In this study local six barley landraces (Hail; Qassem; Taif; Gizan; Bahah and Asser) were collected from two different regions of KSA. Although RAPD's reactions have showed high average of polymorphism (49.8%) comparing with ISSR markers polymorphic average (25.8%), the ISSR results were trustable, repeatable even for intra-specific purposes and could reflect real genetic relationships among barley landraces and correlated with the geographical distribution of them. They are potentially useful markers to identify barley landraces and might be used for cultivar identification. In the crop plants such as barley, the polymorphism rate according to other molecular markers was low. The existence of polymorphic markers was an excellent choice in order to use in different breeding aims.

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