

Full Length Research Paper

Genetic diversity of some Saudi barley (*Hordeum Vulgare* L.) landraces based on microsatellite markers

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Assessment of the genetic variability within barley landraces is fundamental for barley breeding. In this study, 16 simple sequence repeats (SSRs) markers were used to characterize selected six barley landraces from different cultivated regions in Kingdom of Saudi Arabia (KSA) (Gizan, Bahah, Taif, Asser, Qassem and Hail). Amplification of SSRs loci were obtained for 15 primer pairs and only seven among them showed clear polymorphic patterns. These seven primers produce total of 16 alleles with scorable fragment size ranging from approximately 100 to 275 bp. The number of alleles per marker ranged from 1 to 3 with an average of 2.29 alleles per locus. The data generated by these seven primers were sufficient to discriminate the analyzed barley genotypes. Using the unweighed pair-group method using arithmetic averages (UPGMA) cluster analysis, barley landraces were clustered together with respect to their geographical location. These results could be used for barley germplasm management in terms of biodiversity protection and design of new crosses. The present results demonstrate that SSR markers were highly informative and were useful in generating a meaningful classification of barley germplasm.

Key words: Barley, landraces, genetic diversity, molecular markers, microsatellite simple sequence repeat (SSR).

INTRODUCTION

Barley (*Hordeum vulgare*) is an important crop used for animal feed, malt manufactures and human food. Its importance derives from the ability to grow and produce in marginal environments, which are often characterized by drought, low temperature and salinity (Baum et al., 2004). It is second only to wheat as the most important nutritional grain crops grown in low rainfall environments.

In the Kingdom of Saudi Arabia (KSA), barley is cultivated in different geographical regions and considered the most important source for animal feeding. However, due to the highly coast of desalinized water needed for irrigation, barley production in KSA has rapidly decreased since top production of 2 million tons in 1995. Before 1990, local barley landraces were used by farmers in most of the geographical regions of the kingdom.

However, as a result of introduction of imported varieties, the local races were gradually disappeared. Recently, several research programs started to develop new cultivars with high level of salt and drought tolerance to replace the traditional landraces that farmers still grow in most of barley cultivation regions because of its high adaptability and tolerance to salinity. Nevertheless, the continued cultivation of barley landrace and other indigenous crop genetic resources of KSA are potentially threatened and could be lost before they are adequately collected and thoroughly evaluated.

The landraces are the most diverse populations of cultivated plants (Frankel et al., 1995). Effective management and utilization of these resources depends to a large extent on appropriate characterization of their genetic diversity. The genetic diversity among and within landraces makes them a valuable resource as potential donors of genes for the development and maintenance of

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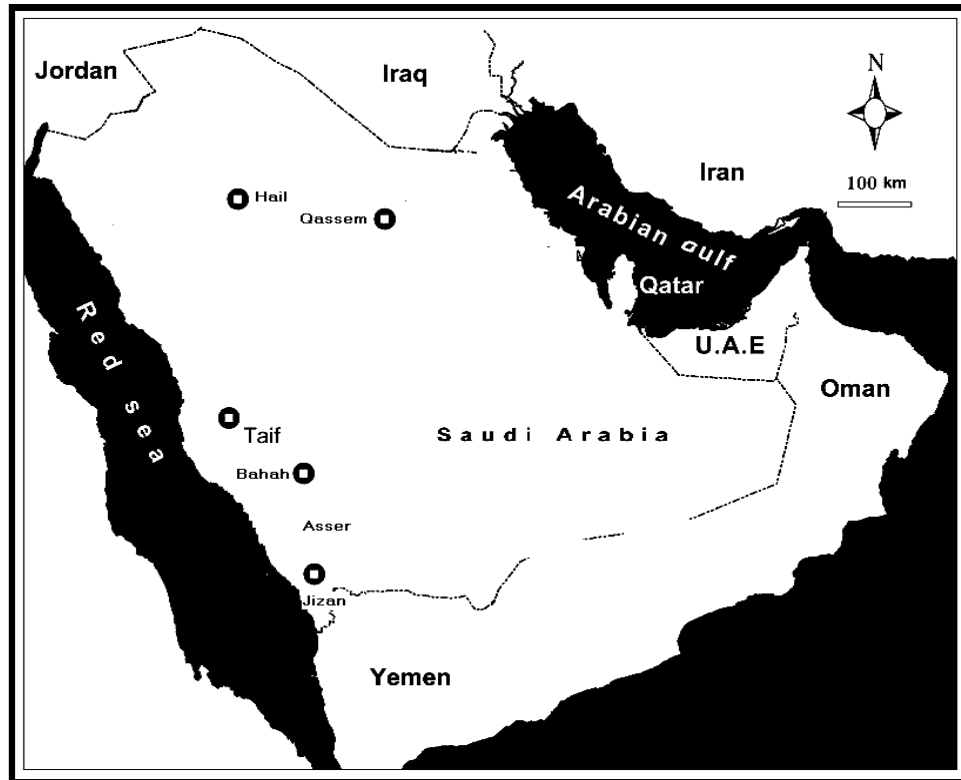


Figure 1. Map of the Kingdom of Saudi Arabia showing the geographical locations of the analyzed barley landraces collection regions.

modern crop varieties and for direct use by farmers (Soleri et al., 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).

A variety of polymerase chain reaction (PCR)-based molecular markers are useful tools for the study of genetic diversity. For detection of genetic variation in barley, different classes of molecular markers were used. However, among these classes, the simple sequence repeats (SSR) or microsatellites (derived from genomic DNA) and amplified fragment length polymorphism (AFLP) have been used separately as well as in combination in many studies (Maestri et al., 2002; Matus and Hayes, 2002; Turpeinen et al., 2003; Nevo et al., 2005; Chaabane et al., 2009). SSRs (Simple Sequence Repeats) or microsatellites are short (mostly 2 to 4 bp) tandem repeats of DNA sequence; their polymorphism originates from a different number of repetitive core motifs present at one locus (Ellegren, 2004). SSRs are codominant, abundant, and informative and their detection can be automated. This makes them an excellent molecular marker system for many types of genetic analyses, including linkage mapping, germplasm surveys, and phylogenetic studies (Liu et al., 1996). Various

repeat motifs (example, di-, tri-, or tetranucleotide units) are reported to occur throughout the genome of most eukaryotic species (Powell et al., 1996).

The available information about the characteristics of barley accessions in KSA is very little. Few studies have been conducted to investigate the genetics relation of the cultivated barley accessions. These studies used only the random amplified polymorphic DNA (RAPD) markers (Hussein et al., 2005) or RAPD and morphological markers (El-Shazly and El-Mutairi, 2006) to analyze the pattern of genetic diversity within barley accessions grown in the KSA.

The objective of this study was to use the SSR markers to investigate the genetic dissimilarity between some selected barley landraces collected mainly from the southwest and central regions of the KSA.

MATERIALS AND METHODS

Six barley landraces collected from six various growing regions of KSA were used in this study. The landraces were named according to the region of their origin from which they were collected (Gizan, Bahah, Taif, Asser, Qassem and Hail). The geographical location of the collection regions are illustrated in Figure 1.

These local landraces were selected for their relatively high tolerance to salt stress to be potentially used in the breeding programs as a donor for salt tolerance genes to the new developed

cultivars.

SSRs analysis

DNA extraction

Seeds were grown in a growth chamber at 27°C under a 12/12 h day/night photoperiod. Genomic DNA was isolated from the leaves collected from 10 to 15-day old seedlings (five plant per genotype) using the plant isolation kit (Jena Bioscience, Germany).

SSR primers

Sixteen (16) microsatellite primer pairs used for genotyping assays were selected on the basis of their chromosomal location described by Ramsey et al. (2000) and Von Korff et al. (2004). Primer names, chromosomal locations, sequences and corresponding annealing temperatures and the amplified fragments are listed in Table 1.

PCR amplification and electrophoresis

PCR amplification was performed in a volume of 20 µl containing approximately 30 ng of template DNA, 1 µm of each forward and reverse primer, 200 Mm of each Dntp, 3 Mm MgCl₂ and 1 U Go Taq DNA Polymerase (Promega) and 10-X Taq polymerase buffer. Reactions were conducted in Eppendorf PCR system (Germany) with initial denaturation step for 5 min at 94°C followed by 35 cycles of 94°C for 1 min, 53 to 63°C (depending on primers, Table 1) for 1 min and 72°C for 2 min; followed by a final extension at 72°C for 5 min. The PCR reaction products were evaluated for polymorphisms on 2% agarose gel. After staining with 1 µg ml⁻¹ ethidium bromide for 30 to 60 min, the gels were photographed by gel documentation system (Gel Doc 2000 Bio-Rad).

Band scoring and cluster analysis

The SSR gel images were scanned using the Gel Doc 2000 Bio-Rad system and analyzed with Quantity One Software v. 4.0.1 (Bio-Rad Laboratories, Hercules, CA USA). The bands were sized and then binary coded by 1 or 0 for their presence or absence in each genotype.

The software SPSS ver. 16 (Statistical Package for the Social Sciences) was used to calculate the pairwise differences matrix and plot the dendrogram among barley cultivars (Yang and Quiros, 1993). Cluster analysis was based on similarity matrix obtained with the unweighted pair-group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

RESULTS

In the present study, 16 primer pairs (Table 1) flanked simple sequence repeats that was employed to investigate the level of polymorphism among the six barley landraces cultivated at different regions of KSA. Fifteen primers produced amplicons and were reproducible. Eight primers showing either amplified monomorphic banding pattern (Bmac0576, Bmac0577, HVITR, HV13GEIII, HVB23, MGB371 and EBmac6245) or complex banding pattern (Bmg0001) were discarded. The SSR marker Bmgtttt1 failed to amplify the expected

PCR fragments. The remaining seven (43.75%) primers which generate polymorphic banding patterns were used for the analysis of genetic diversity. The number of alleles per marker ranged from 2 in four markers (Ebmac0715, MGB391, MGB318 and GMS1) to 3 in three markers (Ebmac0874, Bmag13 and MGB402tt1) with an average of 2.29 alleles per locus. While, six of the polymorphic markers (Ebmac0874, Bmag13, MGB402tt1, Ebmac0715, MGB391, MGB318 and GMS1) showed 100% polymorphism, only the marker MGB391 showed 50% polymorphism (Table 1). Several unique markers that discriminated some landraces from the others were obtained. For example, the marker GMS1 provides a unique marker for the landraces Hail and Qassem (band 185 bp). While, the marker EBmac0874 provides a unique marker for the landraces Hail and Qassem (120 bp) and another unique marker for the landrace Hail (150 bp). Figure 2 shows the polymorphic bands generated by GMS1 and EBmac0874 primers.

Genetic diversity and relationships of barley landraces

To examine the genetic relationships among the six barley landraces under study based on the SSR results, the data scored from the seven primers were compiled and analyzed using the Dice similarity coefficient. The genetic similarity matrices based on the Dice coefficients are shown in Table 2. The average genetic similarity of barley landraces was 78.75. Similarities among the six barley landraces ranged from 60.6% (Hail and Gizan, Qassem and Gizan) to 97.1% (Aseer and Bahah). Figure 3 shows the UPGMA clustering dendrogram based on Dice similarity. The landraces could be grouped into three major groups. The first group contains only Gizan barley. The second group contains Hail and Qassem that are genetically closed to each other with genetic similarity (GS) of 83.3%. The third group contains the three landraces, Asser, Bahah and Taif. Bahah and Asser are very similar genetically (GS = 97.1%) and GS between Bahah and Taif was 88.25%. Remarkably the GS between Taif and Asser was high (91.4%).

The GS between Gizan barley and other five landraces was based on their cultivation regions. Although, Asser, Bahah and Taif showed a high GS (84.8, 81.3 and 75%, respectively) with barley landraces cultivated in the neighbor regions, Hail and Qassem (60.6%) showed less GS between the barley landraces cultivated in the distant regions. Similarly, the GS between both Hail and Qassem barley, and the Taif barley was 80 and 85.7%, respectively, and it was reduced to 74.3, 77.8 and 60.6% with Bahah, Asser and Gizan, respectively.

DISCUSSION

The aim of the present investigation was to explore the

Table 1. Barley SSRs primers (Ramsey et al., 2000; Von Korff et al., 2004), their sequences, the annealing temperature used in the PCR reaction, the chromosomal location of derived loci, the total (T) amplified fragments and the polymorphic (p) amplified fragments.

S/N	Primer	Sequence	Annealing temperature (°C)	Chromosome location	Fragment size	Fragment (T)	Fragment (P)
1	Bmac0576	5'CAATTGTAGCCTAGCTGGTGC 3' 5' GGGTGTATGCAAGTGGGC 3'	53	<u>Bmac0576</u>	149	1	0
2	Bmac0577	5' TCATACAGAAGCCCACACAG 3' 5' TGCATGTTTCATTCTAGACAGG3'	58	<u>Bmac0577</u>	146	1	0
3	Bmg0001	5' GTGTGGTGTAGCAAGGGGT 3' 5' TCACAGGGCACACACACAC 3'	55	<u>Bmg0001</u>	177	1	0
4	Bmgtttt1	5' ACACCAGAGCCTTGACTCGT 3' 5' AGCAGCAACAACGACAACAC3'	63	Bmgtttt1	215	-	-
5	Ebmac0874	5' AACCATTCCTCACCCAGG 3' 5'GTGAATGATGTTGAGGACATTG3'	58	<u>4hac1q2</u>	120-185	3	3
6	Ebmac0715	5' GCGAACATTGTCATGTTAGTA 3' 5' TGTCATGCCAGACCTATG 3'	55	<u>2hac2b3</u>	153-205	2	2
7	MGB391	5'-AGCTCCTTTCCTCCCTTCC-3' 5'-CCAACATCTCCTCCTCCTGA-3'	54	2 (2H)	240-275	2	1
8	HVITR1	5'-CCACTTgCCAAACACTAgACCC-3' 5'-TTCATGCAGATCGGGCCAC-3'	55	3 (3H)	240	1	0
9	Bmag13	5'-AAGGGGAATCAAAATGGGAG-3' 5'-TCGAATAGGTCTCCgAAgAAA-3'	54	3 (3H)	180-210	3	3
10	HV13GEIII	5'-AGGAACCCTACGCCTTACGAG-3' 5'-AGGACCGAGAGTGGTGGTGG-3	56	3 (3H)	180	1	0
11	HVB23D	5'-GGTAGCAGACCGATGGATGT-3' 5'-ACTCTGACACGCACGAACAC-3'	54	4 (4H)	100	1	0
12	MGB402tt1	5'-CAAGCAAGCAAGCAGAGAGA-3' 5'-AACTTGTGGCTCTGCGACTC-3'	55	5 (1H)	240-275	3	3
13	MGB371	5'-CACCAAGTTCACCTCGTCCT-3' 5'-TTATTCAGGCAGCACCATTG-3'	56	6 (6H)	140	1	0
14	Ebmac6245	5'AAAAGCATTCAACTTCATAAGA3' 5'- CAACGCCATCACGTAATA-3'	54	6 (6H)	130	1	0
15	MGB318	5'-CGGCTCAAGGTCTCTTCTTC-3' 5'-TATCTCAGATGCCCTTTCC-3'	55	7 (5H)	100-115	2	2
16	GMS1	5'-CTGACCCTTTGCTTAACATGC-3' 5'TCAGCGTGACAAACAATAAAGG3	55	7 (5H)	150-185	2	2

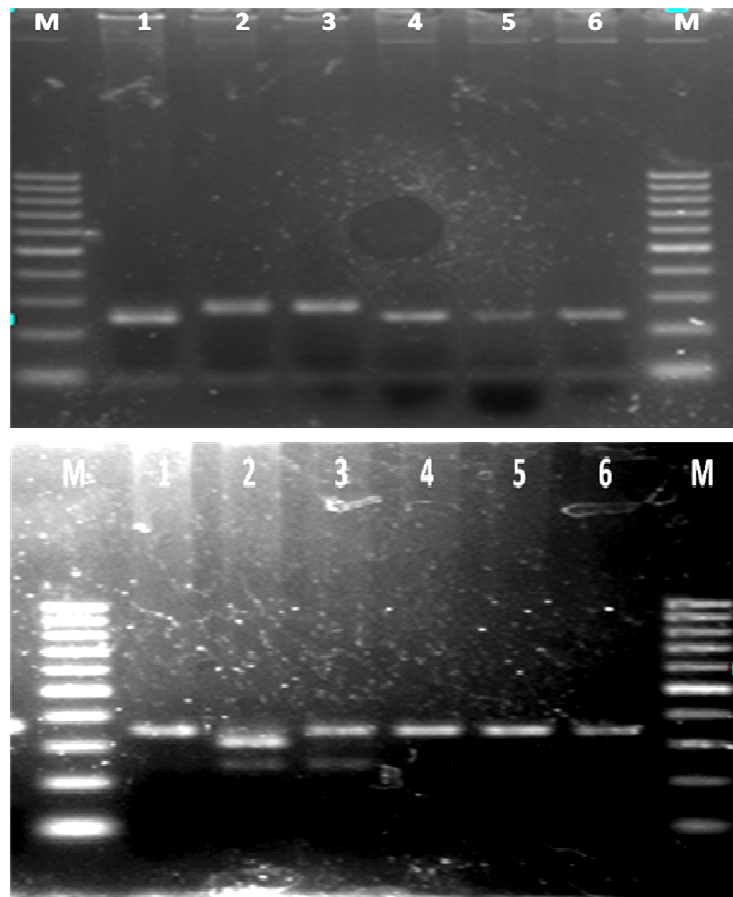


Figure 2. Agarose gel showing the allelic segregation of the SSR markers GMS1 (up) and EBmac0874 (down) in the analysed barley landraces. Lanes 1 to 6 represent Taif, Hail, Qassem, Asser, Baha and Gizan, respectively. M: DNA size marker (50-bp ladder).

Table 2. The genetic similarity matrices among the six barley landraces based on the Dice coefficients.

Barley landrace	Taif	Hail	Qassem	Asser	Bahah	Gizan
Taif	1.00					
Hail	0.800	1.00				
Qassem	0.857	0.833	1.00			
Asser	0.914	0.778	0.778	1.00		
Bahah	0.882	0.743	0.743	0.971	1.00	
Gizan	0.750	0.606	0.606	0.848	0.813	1.00

genetic relationship between six barley landraces cultivated in different geographic regions in KSA using SSR markers. The characterization and assessment of genetic diversity among the barley genotypes would be important for designing breeding strategies for quantitative and qualitative traits. Previously, very few studies were conducted to assess the genetic relationships among the barley genotypes grown in KSA using the morphological and RAPD markers. To our knowledge, the present study is the first to assess the genetic

diversity between barley landraces grown in KSA using the SSR markers. SSR markers are more specific and reproducible markers because of the long primer sequences and high annealing temperature during PCR amplification. We selected 16 SSR primers flanking simple sequence repeats that cover several chromosomal locations in the barley genome. Only seven primers were polymorphic and produced seventeen alleles. Six of the polymorphic markers showed 100% polymorphism and only one marker showed 50%

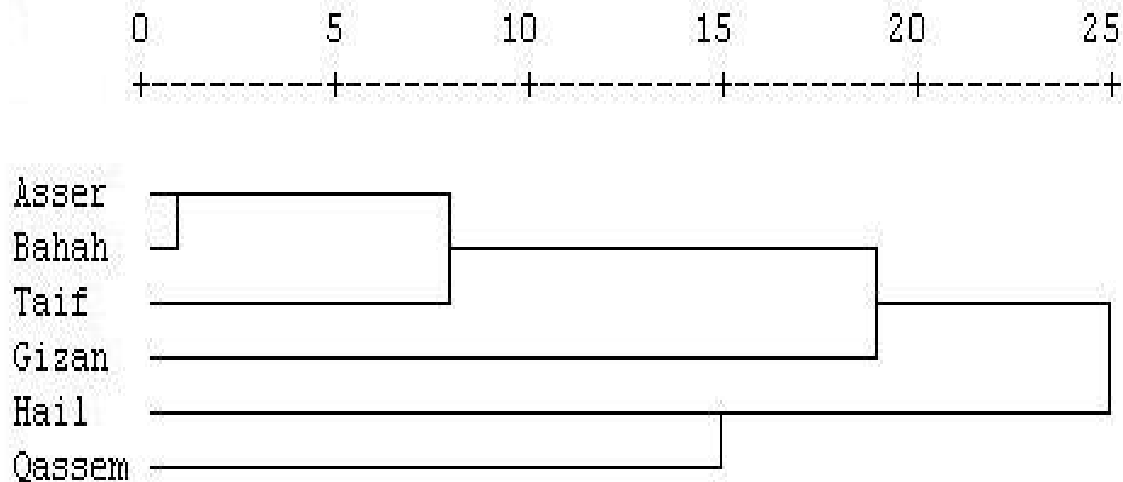


Figure 3. Dendrogram for the six barley landraces constructed from SSRs data analysis using unweighed pair-group arithmetic average similarity matrices computed according to Dice coefficients.

polymorphism (Table 1). The number of alleles per marker ranged from 2 to 3. It was reported that microsatellites are typically multiallelic markers (Matsuoka et al., 2002) with heterozygosity values much higher than those of RFLPs (McCouch et al., 1997).

The landraces Hail and Qassem gave unique fragment of GMS1 with 185 bp and unique fragment of EBmac0874 with 120 bp. Another unique marker (150 bp) for the landrace Hail was obtained with EBmac0874 primers. The unique alleles are important because they may be diagnostic of a particular inbred line or for regions of the genome specific to a particular type of genotype (Senior et al., 1998). Moreover, the occurrence of the unique allele is an indication of the diversity present in a germplasm and its potential as a reservoir of novel alleles for crop improvement (Matus and Hayes, 2002).

The genetic similarity matrices (Table 2) and the UPGMA clustering dendrogram (Figure 2) for the seven SSR markers used in the study suggested different levels of diversity among the six barley genotypes. The landraces could be grouped into three major groups based on their geographical location. The first group contains only Gizan barley that is located at the southeast part of the kingdom. The second group contains the two landraces cultivated in the neighbor regions, Hail and Qassem at the central part of the kingdom. Similarly, the third group contains the three landraces, Asser, Bahah and Taif. A narrow genetic dissimilarity between the landraces clustered in the same group (GS of 83.3% between Hail and Qassem and GS of 97.1 and 91.4% between Asser with Bahah and Taif, respectively). The reduction of the genetic distance between the landraces in the same group may be as a result of the presence of a common ancestor which led to a narrow genetic diversity. It was reported that SSRs are highly variable and therefore able to distinguish closely genetically related plant genotypes (Manifesto et al.,

1999). In contrast, a relatively higher genetic dissimilarity was shown between the landraces of different groups cultivated in different geographic location. Accordingly, the geographical pattern was the main factor for determining genetic variability between the landraces in this study. Low correlation between RAPD markers and the geographic origin was reported in 15 Tunisian barley landraces collected from 8 locations of different bioclimatic zones (Roudha et al., 2010)

In conclusion, this study provides the first analysis of genetic variability among barley landraces in Saudi Arabia using SSR markers. Most of the accessions were closely related. However, the dendrogram generated by the SSR matrix seemed to be effective in discriminating local barleys defined as accessions or populations geographically based. A positive correlation between geographical distance and genetic distance was found. The high similarity between the landraces grown in neighbor is significant, suggesting that they might share common ancestors. In addition, the obtained data confirmed the efficacy of the SSR markers as a highly variable markers that detect the codominant single locus and suitable for distinguishing between the genetically related genotypes. 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 priority in barley improvement.

REFERENCES

- Baum M, Grando S, Ceccarelli S (2004). Localization of quantitative trait loci for dryland characters in barley by linkage mapping. *Challenges and Strategies for Dryland Agriculture*. CSSA Special Publication, 32: 191-202.

- Chaabane R, El Felah M, Ben Salah H, Ben Naceur M, Abdelly S, Ramla D, Nada A, Saker M (2009). Molecular Characterization of Tunisian Barley (*Hordeum Vulgare* L.) Genotypes using Microsatellites (SSRs) Markers. *Eur. J. Sci. Res.* 36: 6-15.
- Ellegren H (2004). Microsatellites: simple sequences with complex evolution. *Nat. Rev. Genet.* 5: 435-445.
- El-Shazly H, El-Mutairi Z (2006). Genetic relationships of some Barley cultivars Based on morphological criteria and RAPD fingerprinting. *Int. J. Bot.* 2: 252-260.
- Frankel OH, Brown AHD, Burdon JJ (1995). The genetic diversity of cultivated plants. In: *The conservation of plant biodiversity*. Cambridge Univ. Press, Cambridge. UK. pp. 39-74.
- Hou YC, Yan ZH, Wei YM, Zheng YL (2005). Genetic diversity in barley from west China based on RAPD and ISSR analysis. *Barley Genet. News.* 35: 9-22.
- Hussein MH, Saker MM, Moghaieb RE, Hussein HA (2005). Molecular characterization of salt tolerance in the genomes of some Egyptian and Saudi Arabian barley genotypes. *Arab. J. Biotech.* 8: 241-252.
- Liu ZW, Biyashev RM, Saghai-Maroo MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theor. Appl. Genet.* 93: 869-876.
- Maestri E, Malceveschi A, Massari A, Marmioli N (2002). Genomics analysis of cultivated barley (*Hordeum vulgare*) using sequence-tagged molecular markers, Estimates of divergence based on RFLP and PCR markers derived from stress-responsive genes and simple Sequence Repeats (SSRs). *Mol. Genet. Geno.* 267: 186-201.
- Manifesto MM, Schlatter A, Hopp HE, Suarez EY, Dubcovsky J (1999). Bread wheat (*Triticum aestivum*) fingerprinting using microsatellites. *Plant and Animal Genome VI, Conference, Town and Country Hotel, San Diego, California, USA, January 17-21.*
- Matsuoka Y, Mitchell SE, Kresovich S, Goodman M, Doebley J (2002). Microsatellites in *Zea* variability, patterns of mutations and use for evolutionary studies. *Theor. Appl. Genet.* 104: 436-450.
- Matus IA, Hayes PM (2002). Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. *Genome*, 45: 1095-1106.
- McCouch SR, Chen X, Panaud O, Temnykh S, Xu Y, Cho YG, Huang N, Ishii T, Blair M (1997). Microsatellite marker development, mapping and application in rice genetics and breeding. *Plant Mol. Biol.* 35: 89-99.
- Nevo E, Beharev A, Meyer RC, Hackett CA, Foster BP (2005). Genomic microsatellite adaptive divergence of wild barley by microclimatic stress in Evolution Canyon. *Israel. Biol. J. Linn. Soc.* 84: 205-224.
- Powell W, Machray GC, Provan J (1996). Polymorphism revealed by simple sequence repeats. In: *Trends in plant science*. (Vol. 1) Elsevier Science Publishers, Amsterdam, The Netherlands. pp. 215-222.
- Ramsay L, Macaulay M, Degli S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvevsson S, Morgante M, Massari A, Maestri E, Marmioli N, Sjakste T, Ganai M, Powell W, Waugh R (2000). A simple sequence repeat-based linkage map of barley. *Genetic*, 156: 1997-2005.
- Raouda A, Karima K, M'barek B, Leila B (2010). Genetic diversity in some Tunisian barley landraces based on RAPD. *Pak. J. Bot.* 42(6): 3775-3782,
- Senior ML, Murphy JP, Goodman MM, Stuber CW (1998). Utility of SSRs for detriming genetic similarities and relationships in maize using an agrose gel system. *Crop Sci.* 40: 226-232.
- Sneath PHA, Sokal RR (1973). In: *Numerical Taxonomy-The principles and practice of numerical classification*. Freeman WH, Company, San Francisco, California, USA. pp. 230-234.
- Soleri D, Smith SE (1995). Morphological and phonological comparisons of two hopi maize varieties conserved in situ and ex situ. *Econ. Bot.* 49: 56-77.
- Turpeinen T, Vanhala E, Nevo E, Nissila E (2003). AFLP genetic polymorphism in wild barley (*Hordeum spontaneum*) populations in Israel. *Theor. Appl. Genet.* 106: 1333-1339.
- Von Korff M, Plümpe J, Michalek W, Léon J, Pillen K (2004). Insertion of 18 new SSR markers into the Oregon Wolfe Barley map. *Barley Genet. News.* 34: 1-4.
- Yang X, Quiros C (1993). Identification and classification of celery cultivars with RAPD markers. *Theor. Appl. Genet.* 86: 205-212.