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Genetic diversity study in some Barley diverse landraces based on RAPD markers in Kurdistan region of Iraq

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ABSTRACT: In this study, the molecular variation of 10 Barley diverse landraces was estimated using random amplified polymorphic DNA (RAPD) markers. These landraces were collected from different bioclimatic Kurdistan region of Iraq. The amplification products varied from 5 to 11 bands ranging between 260 and 2900 bp. On 81 fragments counted, 51 are polymorphic showing a high level of polymorphism (71.8%). The phylogenetic relationship between the studied landraces was evaluated according to phylogenetic tree based on (UPGMA) method that classified barley landraces in 4 homogeneous groups. Among which, the group C included the majority of the landraces with the introduced variety 'Sharazor'. The genetic distance between these landraces is reduced, may be because of the presence of a common ancestor which led to a low genetic diversity. The result of the study indicated that the registered varieties in our country, possesed relatively low genetic variation.

***Keywords*:** Barley, Genetic Variation, Kurdistan, Iraq, RAPD marker

INTRODUCTION

Kurdistan region of Iraq, Iran, Turkey and Syria is one of the principal centers of cereal in the world, and also this region is very rich in both landraces and wild relatives of barley (Forester et al., 2000). The native germoplasm, well adapted to rough and harsh environmental conditions, has been considered to be a large source of genetic diversity and of great importance to varieties' improvement. Among grains crops, barley, *Hordeum vulgare* L., is one of the principal grain crops in the world and is cultivated in all moderate areas (Asif et al., 2005). Wild barleys, *H*. *vulgare* ssp. *spontaneum* and *H*. *vulgare* ssp *agriocrithon*, are the frist gene pool of cultivated barley (*H. vulgare* ssp. *vulgare*). The total number of barley accessions in the Genbanks, comprising redundant materials, is estimated to be about 270,000 (Plucknett et al., 1987). Kurdistan is known to be a genetic diversity center of barley and rich in both landrace and wild relatives of barley (Yang et al., 1987). The wild relatives of barley have been measured to be a large reservoir of genetic diversity. It is known that wild barley populations possess high genetic variation in several useful characters, involving earliness, biomass and yield, protein Component and a high proportion of resistant genotypes against powdery mildew and leaf rust (Nevo et al., 1992). Due to its compatibility and full interfertility with cultivated barley, these wild species have been used as a source of important genes for cultivar development via interspecific crosses (Dávila et al., 1999a). Modern plant breeding and agriculture system have limited the genetic base of cultivated barley (Nevo et al., 1986). Therefore, the modern varieties are more genetically similar and more exposed to pathogens and adverse environmental conditions (Asins et al., 1989). This has advanced the search for new sources of diversity that might be of use in plant breeding programs, and many national and international organizations have stressed the need for the collection, preservation and use of wild-

species relatives of cultivated species and the local varieties (Brown et al., 1990). Assessment of the extent of genetic variability within barley, comprising the wild relatives, is basic for barley breeding and the preservation of genetic resources, and is particularly useful as a general guide in the select of parents for breeding hybrids.
similar any other crop species, the first step in barley improvement is full estimation of the native materials, involving collection, appraisal and molecular characterization of germplasm lines. Knowledge about germplasm diversity and genetic relationships among breeding materials could be an inestimable aid in crop improvement strategies (Abbas et al., 2008). The introduction of molecular markers in plant breeding has presented a valuable tool for the characterization of genetic materials. Among them, RAPD markers have been successfully used in barley germplasm estimation, because of their many advantages. RAPD gained importance due to its simplicity, efficiency and non-requirement of sequence information. RAPD provide, virtually limitless set of descriptors to compare individual plants and populations. With this innovative tool genetic diversity can be estimated and equally it is possible to carry out large scale screening of genetic resources held in gene banks, natural populations, ecosystems and natural reserves with this quick and rapid technique (Tahir et al., 2008). RAPD analysis has been extensively used to document genetic variation in wheat (Cao et al., 1998; Bedo et al., 2000; Gupta et al., 2000), cultivar identification (Malik et al., 1996) and fingerprinting genomes (Welsh and Mcclelland , 1990). The objectives of this study was to detect the genetic diversity of the 10 Barley diverse landraces under study using RAPD-marker technique.

Materials and Method

Plant material and DNA extraction:

A total of 10 barley landraces accessions from 10 locations of Kurdistan region of Iraq were used in this study (Table 1). Total genomic DNA DNA was extracted from a bulk sampling of a minimum of ten individuals for each accession according Aljanabi & Martinez (1997) with slight modifications and then stored at −20 ºC.

Table 1. Landraces' origin, bioclimatic stage and rainfall

Landraces	Origin	Bioclimatic stage	Rainfall (mm)
Kafri 1	Kafri	Sahara	150
Kafri 2	Kafri	Sahara	150
Kalar 1	Kalar	Sahara	170
Kalar 2	Kalar	Sahara	170
Sharazor 1	Darbandikhan	Sub Humid	400
Sharazor 2	Darbandikhan	Sub Humid	400
Sharazor 3	Arbad	Humid inf	600
Shore 1	Arbad	Humid inf	600
Shore 2	Arbad	Humid inf	600
Shahid	Halabcheh	Humid inf	800

Amplification protocol:

A total of fifty arbitrary 10-mer oligonucleotides with arbitrary sequence from Operon (kits A, B, H and R) were used in RAPD analysis (Table 2) The PCR reaction mixture be composed of 20-50ng genomic DNA, 1×PCR buffer, 2.0 mmol/L MgCl2, 100 µmol/L of each dNTP, 0.1 µmol/L primer and 1U *Taq* polymerase in a 25µL volume. The amplification protocol was 94 ºC for 4 min to pre-denature, followed by 45 cycles of 94 ºC for 1 min, 36 for 1 min and 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. Amplification products were fractionated on 1.5 % agarose gel.

Data analysis:

The positions of scorable RAPD bands were changed into a binary character matrix ('1' for the presence and '0' for the absence of a band at a particular position). The genetic dissimilarity (GD) was estimated using Nei & Li method (1979). Based on the dissimilarity matrix, a dendrogram showing the genetic relationships between landraces was builded using the unweighted pairgroup method with arithmetic average (UPGMA) (Sneath and Sokal, 1973) using the software Treecon for Windows version 1.1a. Polymorphic information content (PIC) values were computed for each RAPD primer according to the formula: $PIC = 1-\Sigma(P_{ij})^2$, where P_{ij} is the frquency of the ith pattern showed by the j^{th} primer summed across all patterns revealed by the primers (Botstein et al, 1980).

Results

The results of DNA extraction showed that genomic DNA samples had an optical density ratio as A260/A280 equal to 1.8-2.01. In addition, they had enough concentration about 1773 ng/µl (Fig 1).

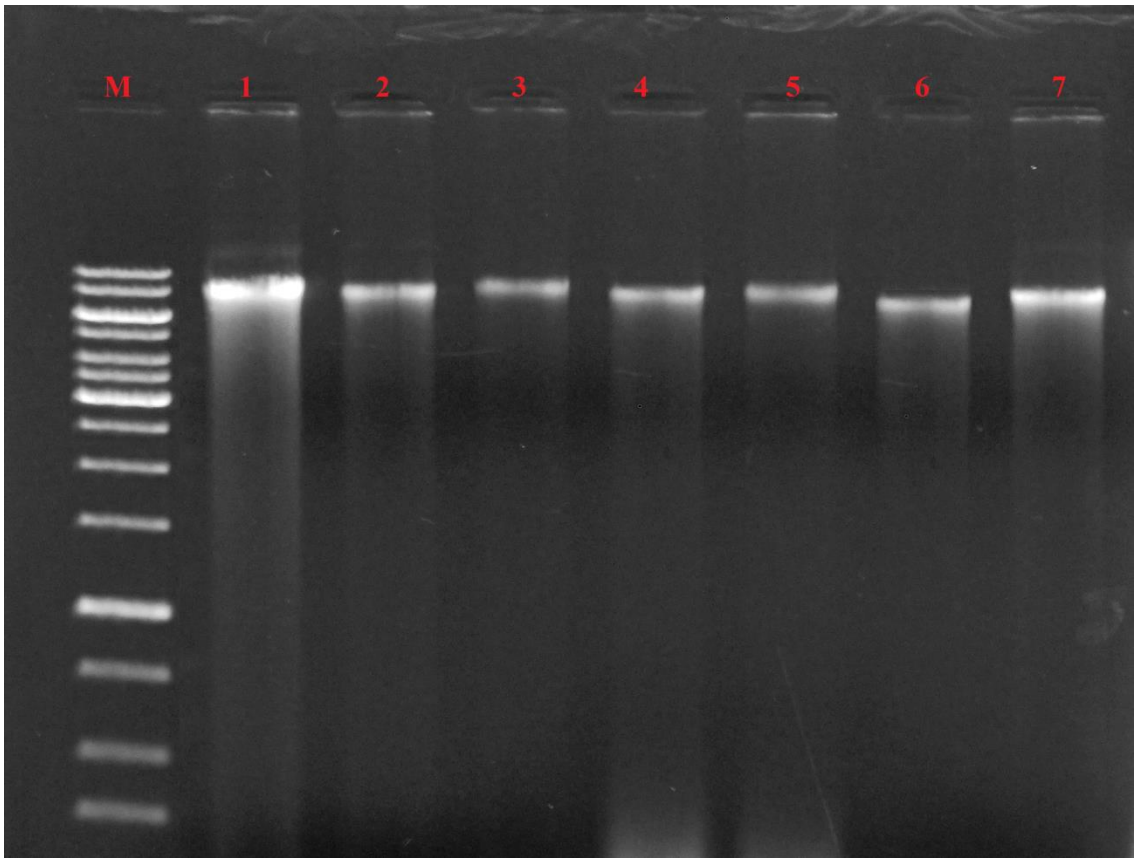

Fig. 1. Electrophoresis bands patterns of DNA extraction. (M): size molecular marker 100 base-pair ladder, (1-6): DNA of Barley landraces genotypes

The results of study showed that the 50 primers screened to evaluate the genetic diversity among the 10 Kurdistan barley landraces, and only 10 primers created polymorphic and reproducible bands. The other 40 primers generated variable, poor amplification products and non-repeatable banding patterns. The results showed the PCR amplification using the efficient 10 primers produced useful marker patterns and analysis gave strong evidence of major genetic differences among barley landraces. On a total of 81 scored DNA fragments 58 bands were polymorphic (71.8%) with an average of 57.8 bands per primer (Table 2). The size of the amplified bands ranged from 260 to 2900 bp. Each primer created 5 to 11 bands. The lowest number of bands (5 fragments) was generated by AP3 and AP8, while the highest (11 fragments) was generated by AP9 of which 11 are polymorphic (Table 2 and Fig. 2).The polymorphic information content (PIC) ranged from 0.74 to 0.89 with an average of 0.87. The lowest and the highest PIC values were recorded for primers AP10 and AP4 respectively.

Table 2. Primer sequences, amplified bands, polymorphic bands and PIC values in RAPD analysis

	Primers	Sequences 5'—3'	Number of total bands	Polymorphic bands	PIC
1	PA 1	GGACCCAACC	9	7	0.87
2	PA 2	GGTCTACACC	7	6	0.89
3	PA 3	GAGAGCCAAC	5	3	0.84
4	PA 4	ACCCGGTCAC	10	8	0.899
5	PA 5	CAGCTCACGA	9	7	0.79
6	PA 6	GGATGAGACC	6	3	0.84
7	PA 7	AGGGCCGTCT	9	4	0.82
8	PA 8	AAGCCCGAGG	5	3	0.84
9	PA 9	CCTGATCACC	11	9	0.86
10	PA 10	GACGCCACAC	10	8	0.74
		Total	81	58	Average= 0.84

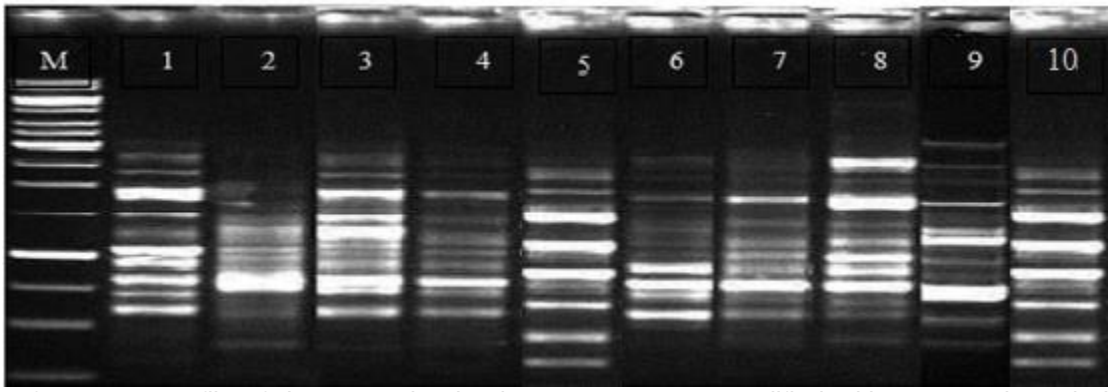

Fig. 2. DNA fragments profile of the 10 Barley landraces genotypes amplified with RAPD primer (Primer AP9). Namely; (M): size molecular marker 100 base-pair ladder. (1): Kafri 1, (2): Kafri 2, (3): Kalar 1, (4): Kalar 2, (5): Sharazor 1, (6): Sharazor 2, (7): Sharazor 3, (8): Shore 1, (9): Shore 2, (10): Shahid.

All the 81 bands, produced from 10 RAPD primers, were used to estimate the genetic distance (RAPD-GD) among the 10 barley landraces (Table 3). The RAPD-GD value ranged from 0.24 to 0.53, with an average of 0.38. The highest genetic distance was discovered between the following landraces' groups (Kafri1 – Kafri 2), while the lowest genetic dissimilarity was observed between (Sharazor 1 - Shore 2). Shahid is a known variety introduced in Kurdistan since 1947 and, because of its high yield seeds it continues to be cultivated in different Kurdistan areas. It has been used by Kurdistanian breeders as an ancestor in their grain improvement programs. That's why it is clustered with some landraces in the same group. The relationships within and between groups were estimated by a UPGMA cluster analysis of GD matrices (Fig 3). It showed that all 10 barley landraces could be distinguished by RAPD markers.

In the dendrogram (Fig. 3) 4 different groups were observed: A, B, C and D. The first group (A) is composed by Shahid which was less related with other groups: (more than 17 % of dissimilarity). The group B are composed by Kafri 1, Kafri 2, Kalar 1 and Kalar 2. The third group (C) gathered the landraces Sharazor 1, Sharazor 2 and Sharazor 3 (34%). In the last group D that this group are composed by Shore 1 and Shore 2. These landraces were more closely related with each other since their dissimilarity percentage is of 28%.

Table 3. Genetic distance of 10 Barley diverse landraces estimated according to Nei and Li(1979) method based on RAPD marker

	1	2	3	4	5	6	7	8	9	10
1	0									
2	0.53	0								
3	0.30	0.37	0							
4	0.41	0.27	0.41	0						
5	0.35	0.37	0.29	0.30	0					
6	0.47	0.40	0.36	0.34	0.34	0				
7	0.38	0.40	0.25	0.42	0.23	0.35	0			
8	0.36	0.34	0.35	0.32	0.30	0.27	0.28	0		
9	0.47	0.36	0.43	0.33	0.24	0.28	0.31	0.40	0	
10	0.38	0.34	0.35	0.30	0.26	0.31	0.38	0.31	0.36	0

1: Kafri 1; 2: Kafri 2; 3: Kalar 1; 4: Kalar 2; 5: Sharazor 1; 6: Sharazor 2; 7: Sharazor 3; 8: Shore 1; 9: Shore 2; 10: Shahid

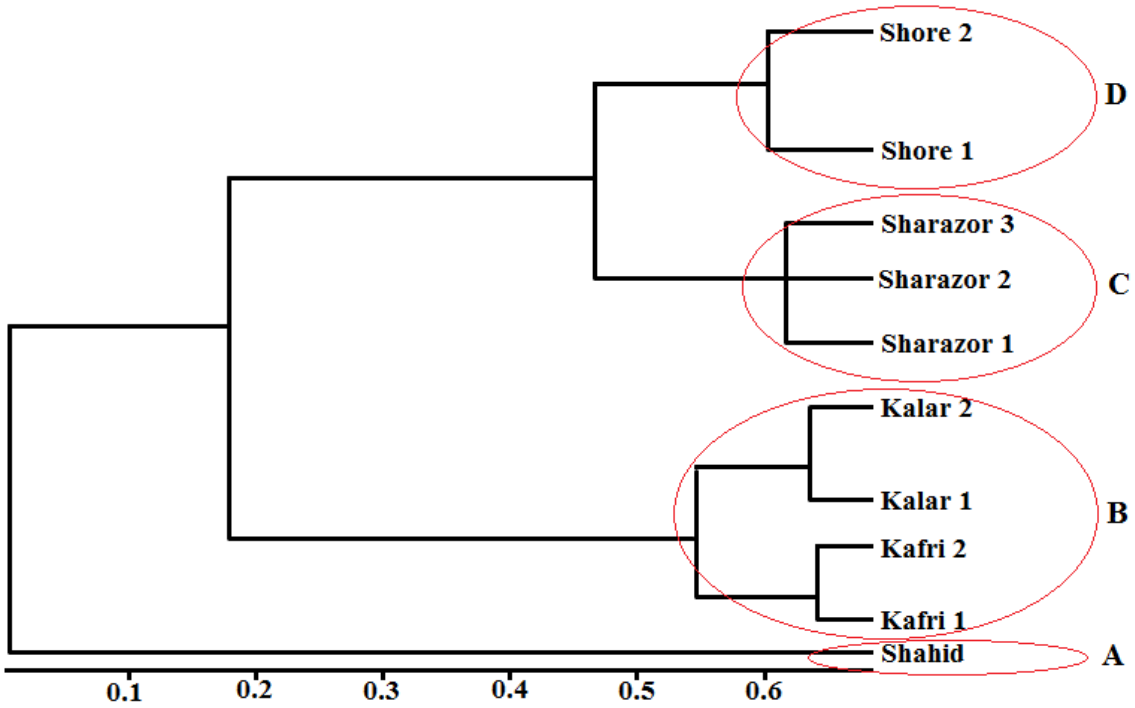

Fig. 3. Dendrogram of 10 barley landraces constructed from RAPD-GD.

Discussion

In this study, genomic DNA was extracted from a bulk sampling for each accession. The results showed that genomic DNA samples had an optical density ratio as A260/A280 equal to 1.8-2.01 that indicates their high quality. In addition, they had enough concentration about 1773 ng/µl The advantages and inconvenient of the bulk analysis have been discussed by Michelmore and coauthor (1991) and Sajida et al. (2009). Bulk analyses are economic and rapid, and it is possible to estimate the genetic variability between accessions, whereas it is not possible to acquire information about the genetic variability within the accessions (Fernández et al., 2002). The number of individual plants bulked for the accessions is an important experimental factor whether the bulked analysis exhibited the genetic relationship between the accessions. Aydogan and Yagdi (2012) found that the bulked samples with 10, 20, 30, 40 and 50 individuals had the same banding pattern. Botstein and coauthor (1980) also found that bulks of 10 to 20 individuals resulted in the same RAPD profiles. Due to its worldwide distribution, the assessment of the genetic diversity among barley germplasm from different countries was performed (Ben Hmida-Ben Salem, 2000; 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). Also, analysis of 10 Kurdistanian of Iran barley cultivars by RAPD markers showed an average genetic diversity of 0.560. In other conducted work on 40 barley Australian cultivars (Pillen et al., 2000), the average genetic diversity was 0.682. Whereas, the genetic diversity between tea populations (Kaundun et al., 2000) was 0.18. In the present study, the average genetic dissimilarity of barley landraces was 0.38. These results proposed that the selected barley landraces have low genetic diversity, despite the high PIC value of primers used. The results showed that the correlation between RAPD markers and the geographic origin is low. Also, the landraces Kafri 1 and Kafri 2 collected from the same region showed an important GD. However, Kalar 1 and Kalar 2 belonging to the same bioclimatic stage showed a low GD. This study agreement with those found in Korean tea populations [30], where no geographical trends were observed among the populations. Similar results had been observed by Lerceteau and coauthor (Sajida et al., 2009). On the other hand, the relationship observed using molecular markers may provide information on the history and biology of cultivars, but it does not necessarily reflect what may be observed with respect to agronomic traits (Métais et al., 2000).This relatively poor relationship observed between RAPD markers and the geographic origin of Kurdistanian barley landraces in this study may be explained by the neutrality of molecular markers compared with adaptive traits under harsh conditions; even if these conditions may influence the cultivar behavior and leads to some adaptation traits such as earliness to avoid water deficit or small ears that will be rapidly filled *etc*…. 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 exhibited the feasibility of a comprehensive effort to determine the relationships among barley landraces using molecular markers. Further collection, evaluation, appraisal**,** and utilization of local germplasm, is clearly a high priority in barley improvement.

CONCLUSION

This study revealed the efficacy of RAPD markers in detecting the polymorphism among barley landraces and establishing relationship among them. Also, the genetic distance between these landraces is reduced, may be because of the presence of a common ancestor which led to a low genetic diversity. The results of the study indicated that the registered varieties in our country, possesed relatively low genetic variation. In addition, the results showed that the correlation between RAPD markers and the geographic origin is low.

REFERENCES

Aljianabi, S.M., Martinez, I. 1997. Universal and rapid salt extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research. 25: 4692 – 4693.

Asif, M., Rehman, M., Zafer, Y. 2005. DNA fingerprinting studies of some wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphic DNA (RAPD) analysis. Pakistan Journal of Botany. 37: 271-277.

Asins, M.J., Carbonell, E.A. 1989. Distribution of genetic variability in a durum wheat world collection. Theoretical and Applied Genetics. 77: 287-294

Abbas, S.J., Shah, S.R.U., Rasool, G., Iqbal, A. 2008. Analysis of genetic diversity in Pakistani wheat varieties by using randomly amplified polymorphic DNA (RAPD) primers American-Eurasian Journal of Sustainable Agriculture. 2(1): 29-33.

Aydogan, E., Yagdi, K. 2012. Study of genetic diversity in wheat (*Triticum aestivum*) varities using random amplified polymorphic dna (RAPD) analysis. Turkish Journal of Field Crops. 17(1):91-95.

Bedo, Z., Szunics, L., Lang, L.U., Szunics, O., Veisz, I., Karsai, G.Y., Vida, P., Szücs, A., Juhasz, M., Gal, SZ., Bencze, M., Megyeri, K., Puskas, C.S., Horvath, H. 2000. Genetic diversity in durum wheat. Annual Wheat Newsletter. Vol:46. http://wheat.pw.usda.gov/gpages/awn/.

Ben Hmida-Ben Salem, W. 2000. Contribution des marqueurs microsatellites et RAPD pourl'études du polymorphisme chez l'orge. Diplômes d'étude approfondies de génétique et biologie moléculaires. *Faculté. Sciences. Tunis. Tunisie*. 80 p.

Botstein, D., White, R.L., Skolnick, M., Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. The American Journal of Human Genetics. 32: 314-331

Botstein, D., White, R.L., Skolnick, M., Davis, R.W. 1980. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. American Journal of Human Genetics. 32:314–331.

Brown, A.D.H., Burdon, J.J., Grace, J.P. 1990. Genetic structure of *Glycine canescens*. A perennial relative of soybean. Theoretical and Applied Genetics. 79: 729-736.

Cao, W., Hucl, P., Scoles, G., Chibbar, R.N. 1998. Genetic diversity within spelta and macha wheats based on RAPD analysis. Euphytica. 104: 181–189.

Dávila, J.A., Loarce, Y., Ramsay, L., Waugh, R., Ferrer, E. 1999a. Comparison of RAMP and SSR markers for the study of wild barley genetic diversity. Hereditas. 131: 5-13.

Fernández, M.E., Figueiras, A.M., Benito, C. 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theoretical and Applied Genetics. 104: 845-851.

Feng, Z.Y., Zhang, Y.Z., Zhang, L.L., Ling, H.Q. 2003. Genetic diversity and geographical differentiation of *Hordeum vulgare* ssp. *spontaneum* in Tibet using microsatellite markers. High. Technology, Letters. 10: 46-53.

Gupta, P.K., Balyan, H.S., Parsad, S., Varshney, R.K., Roy. J.K. 2000. Molecular markers for gene tagging and genetic diversity studies at meerut. Annl. Wheat NewsL, Items from India. 46.

Forester, B.P., Ellis, R.P., Thomas, W.T.B., Waugh, R., Ivandic, V., Tuberosa. R., Talame, V., Teulat-Merah, B., El-Enein, D., Ben Salem, D. 2000. Reaserch developments in genetics of drought tolerance in barly. 8th International Barley Genetics Symposium. 22-27 October. Barley Genetics. 8(1): 233-237.

Kaundun, S.S., Park, Y.G. 2000. Genetic structure of six Korean Tea populations as revealed by RAPD-PCR markers. Crop Science. 42: 594-601.

Nei, M., Li, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases Theoretical and Applied Genetics. 76: 5269-5273

Nevo, E. 1992. Origin, evolution, population genetic and resources for breeding of wild barley, *Hordeum spontaneum* in the fertile Crescent. *In:* Barley: Genetics. Biochemistry. Molecular Biology and Biotechnology (ed PR Shewry) CAB international. Wallingford. 19-143.

Nevo, E., Zohary, D., Beiles, A., Kaplan, D., Storch, N. 1986. Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum*, in Turkey. Genetica. 68: 203-213.

Malik, T.A., Price, A., Wright, D. 1996. Identification of wheat genotypes by random amplified polyorphic DNA technique. *In:* Agricultural Biotechnology, Proc 1st Biotech. Symp, Univ. Agri, Faisalabad, Pakistan. pp: 81-87.

Métais, I., Aubry, C., Hamon, B., Jalouzot, R. 2000. Description and analysis of genetic diversity between commercial bean lines (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics. 1207-1214.

Michelmore, R.W., Paran, I., Kesseli, R.V. 1991. Identification of markers linked to disease resistance genes by bulk segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proceedings of the National Academy of Sciences. 88: 9828-9832.

Pillen, K., Binder, A., Kreuzkam, B., Ramsay, L., Waugh, L., Forster, J., Léon, J. 2000. Mapping new EMBL – derived barley microsatellites and their use in differentiating germen barley cultivars. Theoretical and Applied Genetics. 101: 652-660.

Plucknett, D.L., Smith, N.J.H., Williams, J.T., Murthi, A.N. 1987. Genebanks and the world's food. Princeton University Press, Princeton New Jersey, USA.

Russell, J.R., Fuller, J.D., Macaulay, D., Hatz, B.G., Jahoor, A., Powell, W., Waugh, R. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theoretical and Applied Genetics. 95: 714-722.

Sajida, B., Umar Dahot, M., Imtiaz, A., Khan, A., khatri, A., Naqvi, M.H. 2009**.** Tudy of genetic diversity in wheat (*triticum aestivum* l.) Using random amplified polymorphic dna (RAPD) markers. Pakistan Journal Botani. 41(3): 1023-1027.

Sneath, P.H.A., Sokal, R.R. 1973. Numerical Taxonomy. Freeman, San Francisco.

Tahir, N.A. 2008. Assesment of genetic diversity among wheat varities in sulaimanyah using random amplified polymorphic DNA (RAPD) analysis. Jordan Journal of Biological Sciences. 1(4): 159-164.

Yang, J.L., Yen, C., Lu, B.R., Von, R., Bothmer, R. 1987. The genus *Hordeum* (Gramineae) in China.Variation and distribution. Willdenowia. 16: 479-490.

Welsh, J., Mcclelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research. 18: 7213- 7218.