Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi 10 (2):102-109, 2015
ISSN 1304-9984, *Araştırma Makalesi*

Genetic Divergence in Some Barley (*Hordeum vulgare* L.) Genotypes by RAPD and ISSR Analyses

Murat OLGUN[1] Zekiye BUDAK BAŞÇİFTÇİ[1] N.Gözde AYTER[1] Metin TURAN[2] Onur KOYUNCU[3] Murat ARDIÇ[3] Güleray AĞAR[4] Engin TAKIL[1]

[1]Osmangazi University, Faculty of Agriculture, 26160, Eskişehir/TURKEY
[2]Yeditepe University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering 34755 Ataşehir - Istanbul
[3]Osmangazi University, Faculty of Science, 26480, Eskişehir/TURKEY
[4]Atatürk University, Faculty of Science, 25240, Erzurum/TURKEY
Corresponding author: molgun@ogu.edu.tr

Received: 16. 09.2015, Accepted 20.11.2015

Abstract: The objective of this study was to determine genetic distance between barley genotypes based on RAPD and ISSR analyses. The number of amplified bands of genotypes in the primers ranged 4-11 in RAPD and 7-11 in ISSR. The most polymorphic bands in primer/primer combinations were obtained from OPA-04, OPA-13 and OPH-17 in RAPD, and UBC-808, UBC-820 and UBC-872 in ISSR. Results showed that average polymorphic rate was 86.5%. Performances of cultivars in groups asist possibility in creating genetic variability in barley cultivar development. RAPD and ISSR methods are useful for evaluation of genetic diversity and could be safely used to determine the genetic relationships in barley genotypes.

Key words: Barley, genetic divergence, DNA extraction, genetic polymorphisms, RAPD and ISSR

Bazı Arpa (*Hordeum vulgare* L.) Genotiplerinin Genetik Farklılığının RAPD ve ISSR Analizleri ile Belirlenmesi

Özet: Bu çalışmada RAPD ve ISSR analizleri kullanılarak, arpa çeşitlerindeki genetik farklıların ortaya konması amaçlanmıştır. ISSR ve RAPD analizleri sonucunda, çeşitlerin primer bantlarının; RAPD analizinde 4 ile 11, ISSR analizine ise 7-11 arasında değiştiği belirlenmiştir. Primer ve primer kombinasyonlarının çoğu polimorfik bantların RAPD analizinde OPA-04, OPA-13 ve OPH-17; ISSR analizinde ise UBC-808, UBC-820 ve UBC-872 olduğu belirlenmiştir. Yine elde edilen sonuçlara göre ortalama polimorfik oran % 86.5 olarak tespit edilmiştir. Arpa çeşitleri geliştirilmesi açısından, çeşitlerin genetik farklılıkları belirlenmiş olup, genetik varyabilitenin ortaya konmasına da önemli katkı sağlamıştır. Sonuç olarak RAPD ve ISSR analizleri arpada genetik çeşitliliğin belirlenmesinde faydalı bir yöntem olup, çeşitlerin genetik ilişkisinin ve farklılıklarının belirlenebilmesinde güvenilir bir yöntem olarak kullanılabileceği ortaya konmuştur.

Anahtar kelimeler: Arpa, genetik ayrışma, DNA ektrasyonu, genetik poliforfizm, RAPD ve ISSR

Introduction

Barley has been known as one of the ancient crops and used for animal feed and human food in the world (Nevo 1992). Since ancient times, the importance of barley Cossani et al. 2009). Setter and Waters,2003 Zhou et a., 2007. Barley has wide adaptation ability to different climatic conditions and various environments comprising drought and irrigated environments. Importance of barley production is tremendously increasing with increasing need to feed animal production

and industrial purpose (Sayre et al., 1997; Jayahar, 2012). To meet increasing demands will only possible to develop barley genotypes with higher quality and yielding, disease and pest resistant and high adaptability and this phenomena could be overcome by comprehensive and multi-purpose breeding programs having a vast genetic pool playing vital role to develop novel barley genotypes (lit Poehlman, 1987; Kang, 1990; Mohammed, 2009). Besides, classic and biotechnical techniques have been efficiently used in plant breeding programs and DNA markers are ordinarily used to allow cultivar identification and fingerprint of genomes in crops and (lit Karp et al., 1997; Mukhtar *et al*., 2002). Mohapatra et al. 2003; Motawei et al., 2007. RAPD and ISSR are rapid and efficient applications in evaluation, characterization of genetic material. They create opportunity to segregate features and diversities of genetic resources, to show cultivar identification and fingerprint of genomes in cereals lit Welsh and McClelland,1990; Cao et al.1998; Malik et al., 1996; Gupta et al. 2000; Naghavi et al. 2004; Tahir, 2008). Deshmukh et al., 2012 Yang et al., 1996; Karaca and Izbirak, 2008). This study is aimed to determine genetic distance between barley genotypes based on RAPD and ISSR analyses, and will be helpful in future for genetic studies to lead development of novel barley genotypes in breeding programs.

Materials and Methods

This study was carried out in greenhouse and laboratory conditions at Osmangazi University, Agricultural College in Eskişehir, Turkey. Seeds were sown in PVC containers (0,75 m width, 1 m length, and 0,75 m height) containing 80 kg of loamy textured soil (33,4 % sand, 36,6 % silt, and 30,0% clay), and plants were allowed to 15 cm height. Leaf samples from barley genotypes were randomly selected plants were collected and stored at −20 °C until use. CTAB method (Saghai-Maroof et al, 1984), providing better quality and quantity of DNA was used to isolate genomic DNA of genotypes then genomic DNA extracted was subjected to PCR amplification using RAPD and ISSR markers. Twelve barley genotypes were used and information of them was given in Table 1.

RAPD and ISSR techniques, used to determine genetic distances between genotypes included four parts; DNA extraction, PCR processes, electrophoresis and analysis of data. **DNA extraction:** Genomic DNA was extracted from powdered leaf materials using the Qiagen DNA exraction kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The purity and quantity of genomic DNA was determined spectrophotometrically and confirmed using 0.8% agarose gel electrophoresis against known concentrations of unrestricted lambda DNA. **RAPD amplification:** 45 primers had been used to generate RAPD profiles. PCR amplification reactions were carried out in thirty µl final volume of reaction mixture containing 10x Buffer 3.0 µl, dNTPs (10mM) 1.2 µl, magnesium chloride (25mM) 1.2 µl, primer (5µM) 2.0 µl, *Taq* polymerase (5unit) 0.4 µl, water 19.2 µl sample DNA 3.0 µl (100ng/ µl). The thermalcycler (Eppendorf Company) was DNA amplification. Five primers were chosen for ISSR analyses of genetic diversity, based on band reproducibly (Table 1). PCR reactions were carried out using a single primer at a time, in 25 mL reaction mixture containing 40 ng of template DNA, 1_ reaction buffer, 200 mM of each of the four dNTPs, 1 U of Taq DNA polymerase, 1.5 mM MgCl2 and 0.5 mM of primer. Amplification was performed using a thermal cycler programmed for an initial denaturation step of 5 min at 94ºC followed by 35 cycles of 45 s at 94ºC, 1 min at the specific annealing temperature and 1 min at 72ºC, ending with a final extension step of 7 min at 72ºC. The PCR products of ISSR markers were resolved by electrophoresis on 1.5% agarose gels. **Electrophoresis:** The PCR products (27 µl) were mixed with 6x gel loading buffer (3 µl) and loaded onto an agarose (1.5% w/v) gel electrophoresis in 0.5XTBE (Tris-Borate- EDTA) buffer at 70 V for 150 min. The gel was stained in ethidium bromide solution (2 µl Etbr/100ml 1xTBE buffer) for 40 min and visualized

under UV in Bio Doc Image Analysis System with Uvisoft analysis package (Cambrige, UK).

Data analysis: PCR products were scored as presence (1) and absence (0) of band for each genotypes and analyzed. Data were used to calculate using Nei-Li's similarity index (Nei and Li, 1979) from which a UPGMA dendrogram was constructed. All of the experiments in this study are repeated at twice.

Table 1. Information of plant characteristics in barley genotypes.
Çizelge 1. Arpa çeşitlerine ait bitki özellikleri

Variety *Çeşitler*	Spike Traits *Başak Tipi*	Plant Height (cm) *Bitki Boyu (cm)*	Growth Habit *Gelişim Tabiatı*	Protein Content (%) *Protein Oranı (%)*	Test Weight (kg/hl) *Hektolitre Ağırlığı (kg/hl)*	Thousand Seed Weight (g) *Bin Tane Ağırlığı (g)*
Konevi-99	2 Rows White	100,11±4,34	Alternative	12,14±1,12	62,47±4,21	36,37±2,44
Kalaycı-97	2 Rows White	85,15±3,87	Alternative	11,56±0,22	65,32±2,54	33,15±2,29
Beyşehir-98	2 Rows White	90,35±6,23	Alternative	12,05±2,54	64,48±3,45	34,76±3,06
Sladoran	2 Rows White	86,85±2,88	Alternative	11,27±2,76	62,37±2,45	33,02±3,04
Bolayır	2 Rows White	95,75±3,98	Alternative	10,61±1,88	64,88±1,54	36,18±2,43
Harman	2 Rows White	84,45±4,01	Alternative	11,35±0,44	61,54±2,23	33,61±1,68
Çıldır- 02	2 Rows White	75,23±2,33	Alternative	10,92±0,43	61,04±3,11	34,78±2,17
İnce-04	2 Rows White	98,44±2,67	Alternative	10,41±1,43	62,12±2,15	32,19±3,46
Karatay-94	2 Rows White	86,10±3,14	Alternative	11,35±2,12	67,77±2,98	33,86±2,35
Kıral- 97	6 Rows White	89,60±5,21	Alternative	13,04±1,25	66,35±3,34	38,41±1,98
Erginel- 90	6 Rows White	88,55±3,18	Alternative	12,71±1,78	65,31±2,76	39,37±2,68
Martı	6 Rows White	91,67±2,56	Alternative	12,83±2,02	66,76±3,35	40,26±3,56

Results and Discussion

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop after wheat, maize, rice, and is commonly used as not only animal feed but malting (Drew and Sisworo, 1977; Bingru et al., 1994;).

RAPD and ISSR analyses are DNA-based markers, lead huge amount of polymorphism and fingerprint features, measure genetic diversity for evaluating genetic diversity (Vierling and Nguyen 1992; Qian et al. 2001), and they have been commonly used in plant breeding programs, variety identification (Sofalian et al., 2009; El-Assal and Gaber, 2012; Deshmukh et al., 2012).

RAPD analysis

Results of our RAPD analysis are summarized in Table 2. Twelve primers have generated reproducible and polymorphic bands. A total of 95 bands were recorded with 89 polymorphic. In total, 93.6 % of the bands were polymorphic. The size of the ampliconsrange between 250 bp to 3200 bp. Primers OPA- 4 gave the highest number of RAPD products (11). Primers OPBB- 3 gave the lowest number of RAPD products (4) (Table 2). Dendogram of twelve barley genotypes by RAPD was given in Figure 1. A dendrogram constructed according to RAPD data (Figure 1) of 12 barley genotypes divided them into three main clusters. The first cluster included Martı, Erginel-90 and Kral. The second cluster, having five genotypes, was also

divided into two subclusters: the first subcluster consisted of Beysehir-98, Karatay-94 and Konevi-99. The second subcluster only consisted of Kalaycı-97. The third cluster had two subclusters: the first included Ince-04, Çıldır-02, Bolayır and Harman. The second subcluster had only Sladoran. The greatest similarity was observed between Çıldır-02 and Bolayır (0.164), the greatest dissimilarity was observed between Beyşehir-98 and Kral-97 genotypes (0. 812). It was interesting result that six-row barleys, Kral-97, Erginel-90 and Martı represented similar genetic polymorphism (Figure 1).

Table 2. Details of banding pattern revealed through RAPD and ISSR markers
Çizelge 2. RAPD ve ISSR markörlerine ait protein bantlarının özellikleri

Primer/primer Combination *Primer/primer Kombinasyonu*	Sequence (5'–3') *Sekans (5'–3')*	Length of Amplified Bands *Bant Uzunluğu*	No of Bands *Bant Numarası*	No of Polymorphic Bands *Polimorfik Bant Numarası*	Polymorphism Ratio (%) *Polimorfizm Oranı (%)*
		RAPD			
A-1	AGTCAGCCAC	500– 1800	7	6	100
OPK19	CACAGGCGGA	750–3000	9	8	88.8
OPBB- 03	TCACGTGGCT	250– 2000	4	4	100
B-20	GGACCCTTAC	500-2400	7	7	100
OPA– 04	AATCGGGCTG	750-2800	11	10	90.9
OPA-13	CAGCACCCAC	500– 2700	10	10	100
OPH- 17	CACTCTCCTC	250– 1800	10	9	90
OPW– 6	AGGCCCGATG	400-2500	7	7	100
OPL09	TGCGAGAGTC	400– 3000	8	7	87.5
OPY06	AAGGCTCACC	500– 3200	8	8	100
OPY13	GGGTCTCGGT	600– 2500	9	8	88.8
OPW– 17	GTCCTGGGTT	600-2200	5	5	100
Total/*Toplam*		250-3200	95	89	93.6
		ISSR			
UBC-808	(AG)8C	500- 2100	8	8	100
UBC- 820	(GT)8C	250-2800	11	10	90.9
UBC- 872	(GATA)4	400- 3000	9	9	99.9
UBC-842	GA)8YG	500- 2600	6	5	83.3
UBC- 825	(AC)8T	600- 2500	7	7	100
Total/*Toplam*		250-3000	41	39	95.1

*Not repeatable; **type of degeneratenucleotide: Y = pYrimidine (C, T); R = puRine (A, G).
**Tekrarlamayan; **Dejenere nükleotit tipi: Y = pYrimidin (C, T); R = püRin (A, G).*

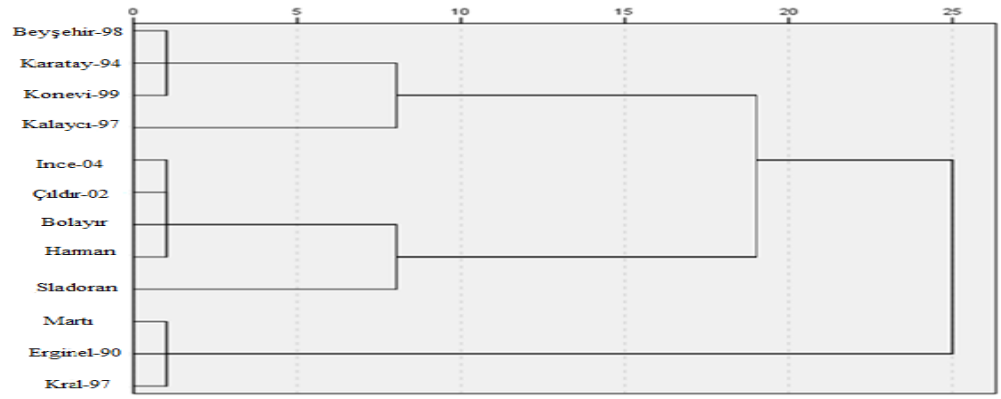

Figure 1. UPGMA clustering for 12 barley genotypes based on RAPD markers
Şekil 1. On iki arpa çeşidinde RAPD markörlerine dayalı UPGMA cluster analizi

It was stressed that RAPD method has been used for measuring genetic diversity in cereals particularly in barley and wheat (Vierling and Nguyen 1992; Qian et al. 2001). RAPD was also successfully used in genetic variations in wild populations of four species of the genus of *Hordeum* (De Bustos et al. 1998).

ISSR analysis ISSR (inter-simple sequence repeat) method is based on dinucleotide, tetra nucleotide or penta nucleotide repeats has been used in cereals (Nagaoka and Ogihara 1997). Twelve barley genotypes were surveyed by using 5 ISSR primers. A total of 41 bands were identified, of which 39 were polymorphic (95.1%) with a minimum of 6 (UBC842) and a maximum of 11 (UBC 820) bands per primer (Table 2). The size of amplified fragments ranged from 250 to 3000 bp with an average of 8.2 fragments per primer. The percentage of polymorphic bands produced by each primer ranged from 83.3 % (UBC842) to 100 % (UBC-808, UBC- 825). Dendrogram based on UPGMA analysis of the ISSR data is shown in Figure 2.

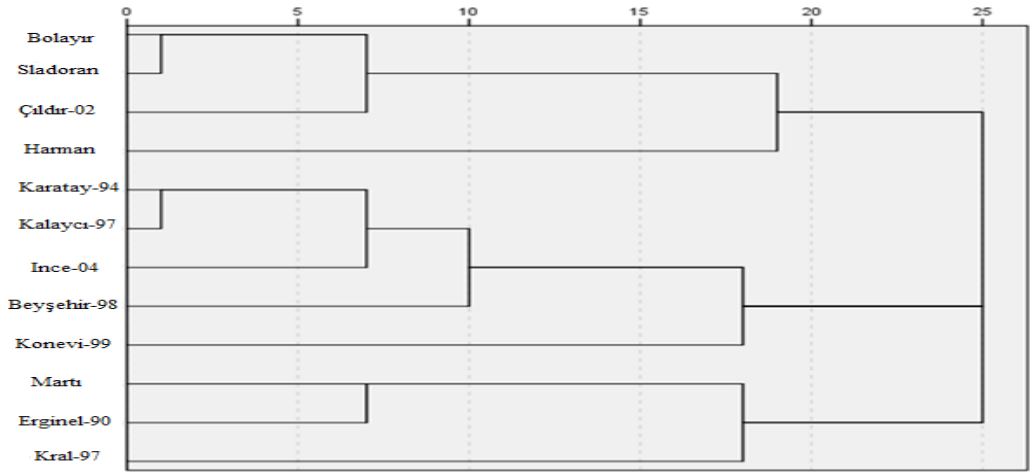

Figure 2. UPGMA clustering for 12 barley genotypes based on ISSR markers
Şekil 2. On iki arpa çeşidinde ISSR markörlerine dayalı UPGMA cluster analizi

The twelve samples were placed into three clusters. Cluster I had two subclusters: the first subcluster comprised Bolayır, Sladoran, Çıldır-02 genotypes; whereas the second one had only Harman genotype. Cluster II also constituted of two subcluters: subcluster I had Karatay-94, Kalaycı-97, Ince-04, Beyşehir-98 genotypes. Subcluster II had only Konevi-99. Cluster III had all six-row barley genotypes, and occupied two subcluster: subcluster I constituted of Martı, and subcluster II had Erginel-90 and Kral-97 genotypes. The greatest similarity was observed between Karatay-94 and Kalaycı-97 genotypes (0.189), the greatest dissimilarity was observed between Bolayır and Kral-97 (0. 867). The UPGMA cluster was constructed using a combination of data from the RAPD and ISSR markers was shown in Figure 3. The twelve barley genotypes were classified into two major groups. Cluster I had two subclusters: the first subcluster had Karatay-94, Konevi-99, Beyşehir-98, Çıldır-02 and Kalaycı-97. Subcluster II constituted of Sladodan, Harman and Bolayır genotypes. Cluster II included subluster I with Martı and Ince-04 genotypes; subcluster II with Kral-97 and Erginel-90 genotypes (Figure 3). The similarity matrix values of barley genotypes varied between 0.162 and 0.869. The greatest similarity was observed between Çıldır-02 and Kalaycı-97 genotypes with 0.152, the greatest dissimilarity was found between Karatay-94 and Erginel-90 with 0. 869. Our results showed that average polymorphic rate was 86.5%. Performances of cultivars in groups asist possibility in

creating genetic variability in barley cultivar development. RAPD and ISSR methods are useful for evaluation of genetic diversity and could be safely used to determine the genetic relationships in barley genotypes.

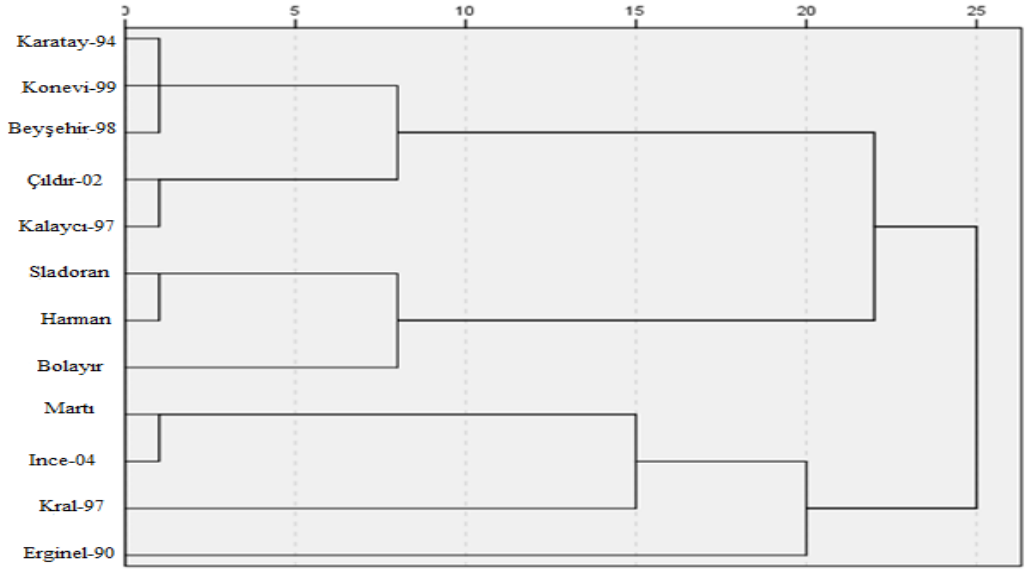

Figure 3. UPGMA clustering for 12 barley genotypes based on RAPD and ISSR markers
Şekil 3. On iki arpa çeşidinde RAPD ve ISSR markörlerine dayalı UPGMA cluster analizi

References

Bingru, H., J.W. Johnson, S. Nesmith, and D.C. Bridges. 1994. Growth, Physiological and Anatomical Rresponses of Two Wheat Genotypes to Waterlogging and Nutrient Supply. Journal of Experimental Botany 45(2): 193-200.

Cao, W., P. Hucl, G. Scoles, and R.N. Chibbar. 1998. Genetic Diversity within Spelta and Macha Wheats Based on RAPD Analysis. Euphytica 104: 181–189.

Cossani C. M.,. G.A. Slafer, and R. Savin. 2009. Yield and Biomass in Wheat and Barley Under A Range of Conditions in a Mediterranean Site., Field Crops Research 112: 205–213.

De Bustos, A., C. Casanova, C. Soler, and N. Jouve. 1998. RAPD Variation in Wild Populations of Four Species of The Genus Hordeum (Poaceae), Theor Appl Genet 96 : 101-111

Deshmukh, R., N.S. Tomar, N. Tripathi, and S. Tiwari. 2012. Identification of RAPD and ISSR Markers for Drought Tolerance in Wheat (*Triticum aestivum* L.). Physiol Mol Biol Plants. 18 (1): 101–104.

Drew, M.C., and E.J. Sisworo. 1977. Early Effects of Flooding on Nitrogen Deficiency and Leaf Chlorosis in Barley. New Phytol. 79: 567-571.

El-Assal, S.E.D.,, and A. Gaber. 2012. Discrimination Capacity of RAPD, ISSR and SSR Markers and of Their Effectiveness in Establishing Genetic Relationship and Diversity Among Egyptian and Saudi Wheat Cultivars. Am J Applied Sci. 9: 724-735.

Gupta, P.K., H.S. Balyan, M. Parsad, R.K. Varshney, and J.K. Roy. 2000., Molecular Markers for Gene Tagging and Genetic Diversity Studies at Meerut. Annl Wheat Newsl. Items from India: 46.

Jayahar, R.P. 2012. Physiological and Anatomical Implications of Salinity on Rice as a Semi-acquatic Species. Cambridge Scholars Publishing: 1-5.

Kang, M.S. 1990. Using Genotype by Environment Interaction for Crop Cultivar Development. Department of

Agronomy. Advance in Agronomy. 62: 199-252.

Karaca, M., and A. Izbirak. 2008. Comparative Analysis of Genetic Diversity in Turkish Durum Wheat Cultivars Using RAPD and ISSR Markers. J Food Agric Environ. 6: 219-225.

Karp, A., S. Kresovich, K.V. Bhat, W.G. Ayad, and T. Hodgkin. 1997. Molecular Tools in Plant Genetic Resources Conservation: a Guide to The Technologies. In: IPGRI Technical Bulletin No. 2. International Plant Genetic Resources Institute, Rome, Italy

Malik, M. A., M.H. Rasheed, and A. Razzaq. 1996. Row Spacing Study on Two Wheat Variety under Rainfed Conditions. Sarhad J Agric. 12(1): 31-36.

Mohammed, M.I. 2009. Genotype X Environment Interaction in Bread Wheat in Northern Sudan Using AMMI Analysis. American-Eurasian J Agric and Environ Sci. 6(4): 427-433.

Mohapatra, T., S.S. Krishanpal, S.C. Singh, R.Swain, K. Sharma, and N.K. Singh. 2003. STMS Based DNA Fingerprints of The New Plant Type Wheat Lines. Current Science., 84(8): 1125-1129.

Motawei, M.I., A. A. Al-Doss, and K.A. Moustafa. 2007. Genetic Diversity Among Selected Wheat Lines Differing in Heat Tolerance Using Molecular Markers. J Food Agric Environ. .5(1) : 180-183.

Mukhtar, M.S., M. Rahman, and Y. Zafar. 2002. Assessment of Genetic Diversity Among Wheat (*Triticum aestivum* L.) Cultivars From A Range of Localities Across Pakistan Using Random Amplified Polymorphic DNA (RAPD) Analysis. Euphytica. 128: 417-425.

Nagaoka, T., and Y. Ogihara. 1997. Applicability of Inter-simple Sequence Repeat Polymorphisms in Wheat for Use As DNA Markers in Comparison to RFLP and RAPD Markers. Theor Appl Genet. 94: 597-602.

Naghavi, M. R., M. Mardi, H. A. Ramshini, and B. Fazelinasab. 2004. Comparative Analyses of The Genetic Diversity Among Bread Wheat Genotypes Based on RAPD and SSR Markers. Iranian J Bio. 2(3): 195-202.

Nei, M., and W.H. Li. 1979. Mathematical Model for Studying Genetic Variations in Terms of Restriction Endonucleases. Proceedings of the National Academy of Sciences. 76: 5269-5273.

Nevo, E. 1992. Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. University of Haifa: 19-43.

Poehlman, J.M. 1987. Breeding Wheat and Triticale. Pp. 220-239, In J. M. Poehlman, ed. Breeding Field Crop. AVI Publishing Company Inc., Westport.

Qian, W., S. Ge, and· D.Y. Hong. 2001. Genetic Variation Within and Among Populations of a Wild Rice Oryza Granulata from China Detected by RAPD and ISSR markers. Theor Appl Genet. 102:440–449

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen, R.W. Allerd. 1984. Ribosomal Spacer Length Polymorphism in Barley: Mendelian Inheritance, Chromosomal Location and Dynamics. Proc Natl Acad Sci. 81: 8014–8019

Sayre, K.D., S. Rajaram, and R.A. Fischer. 1997. Yield Potential Progress in Short Bread Wheats in Northwest Mexico. Crop Sci. 37: 36–42

Setter, T.L.,and I. Waters. 2003. Review of Prospects for Germplasm Improvement for Waterlogging Tolerance in Wheat, Barley and Oats. Plant Soil. 253: 1–34.

Sofalian, O., Chaparzadeh,N., and M. Dolati. 2009. Genetic Diversity in Spring Wheat Landraces from Northwest of Iran Assessed by ISSR Markers. Notul Bot Hort Agric. Cluj-Napoca. 37: 252-256.

Tahir, N.A., 2008. Assesment of Genetic Diversity Among Wheat Varities in Sulaimanyah Using Random Amplified Polymorphic DNA

(RAPD) Analysis. Jordan J Bio Sci. 1(4): 159-164.

Vierling, R.A., and H. T. Nguyen. 1992. Use of RAPD Markers to Determine the Genetic Diversity of Diploid, Wheat Genotypes. Theo Appl Gen. 84(7-8): 835-838.

Welsh, J., and M. McClelland. 1990. Fingerprinting Genomes Using PCR with Arbitrary Primers. Nucleic Acids Res. 18: 7213-7218.

Yang, W., A.C. Olivera, I. Godwin, K. Schertz, and J.L. Bennetzen. 1996. Comparison of DNA Marker Technologies in Characterizing Plant Genome Diversity: Variability in Chinese Sorghums. Crop Sci. 36: 1669-1676.

Zhou, M.X., H.B. Li, and N.J. Mendham. 2007. Combining Ability of Waterlogging Tolerance in Barley. Crop Sci. 47: 278–284.