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Identification of Genetic Divergence in Some Bread Wheat Varieties By Rapd and Issr Analyses

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Abstract: The objective of this study was to determine genetic distance between some wheat varieties based on RAPD and ISSR analyses. The number of amplified bands of genotypes in the primers ranged 3-10 in RAPD and 6-12 in ISSR. The most polymorphic bands in primer/primer combinations were obtained from OPBA-03, OPL-15 and OPY-13 in RAPD, from UBC810 in ISSR. Varieties were classified about in three groups based on the dendogram results. Yunus and Altay-2000, Nacibey and Sultan-95, Harmankaya and Soyer were found to be close related varieties, whereas distant-related varieties were Alpu-01 and Nacibey. RAPD and ISSR are efficiently used to evaluate genetic variation, to determine genetic diversities and improvement and development of novel varieties.

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

Bazı Ekmeklik Buğday Çeşitlerinde Rapd ve Issr Analizleriyle Genetik Farklılıkların Belirlenmesi

Özet: Bu çalışmada, bazı buğday çeşitlerinin arasındaki RAPD ve ISSR analizine dayalı genetik mesafelerin belirlenerek ayrımının yapılması amaçlanmıştır. Araştırma sonuçlarına göre, primerler genotipleri güçlendirilmiş bantların sayısı RAPD' de 3-10, ISSR' de 6-10 arasında değiştiği belirlenmiş olup, primer/primer kombinasyonları içerisinde RAPD'de OPBA-03, OPL-15 ve OPY-13'den; ISSR'de UBC810 elde edildiği ortaya konmuştur. Dendogram sonuçlarına göre çeşitler göre üç grup altında sınıflandırılmıştır. Yunus, Altay-2000, Naci Bey, Sultan-95, Harmankaya ve Soyer çeşitlerinin uzak ilişkili; Alpu-01 ve Nacibey çeşitlerinin ise yakın ilişkili çeşitler olduğu tespit edilmiştir. Yine RAPD ve ISSR yöntemlerinin genetik varyasyonu değerlendirmek, yeni çeşitlerin geliştirilmesi ve sınıflamasında kullanılabilir yöntemler olduğu belirlenmiştir.

Anahtar kelimeler: Buğday, genetik farklılık, DNA ekstraksiyonu, genetik polimorfizm, RAPD ve ISSR analizleri

Introduction

Bread wheat (*Triticum aestivum* L.) as a member of Cereal family is so important crop; multipurpose use and nutritional value make bread wheat strategic and staple food in the world (Asif et al., 2005; Anonymous, 2011). Acreage (almost 230 million hectare of the total cultivated land) and production (almost 700 million tons) explain that wheat is desired crop in the international trade (Shashikala, 2006; Anonymous, 2012). Dizzying increase in world population, alarming decrease in resources for wheat

production will increase demands for wheat (Anonymous, 2012). Supplying necessary food to growing world population will need to increase wheat production. Besides, development of bread wheat varieties studies with better quality, higher yielding, disease- and pest-resistant and high adaptability could probably assist to overcome these difficulties (Poehlman, 1987; Kang, 1990; Mohammed, 2009). Huge differences in genetic material are so vital novel varieties that play key role achieving yield production (Evans and Fischer, 1999). Successful breeding programs are closely related to breadth and diversity of genetic material and both conventional and biotechnical procedures are extensively used in plant breeding programs (Karp et al., 1997; Mukhtar et al., 2002). DNA markers are common biotechnical methods to assist successive applications in plant growth (Mohapatra et al., 2003). Evaluation and revealing of genetic base by DNA markers are the main step for germplasm characterization. RAPD and ISSR with rapid and efficiently applications have been extensively used in variety evaluation (Deshmukh et al., 2012). RAPD could provide opportunity to compare individual crops and genetic diversity could be assigned with large scale to segregate features of genetic resources in different aims and applications (Malik et al., 1996; Gupta et al., 2000; Naghavi et al., 2004; Tahir, 2008). It was mentioned that RAPD analysis has been extensively used in cultivar identification and fingerprint of genomes in cereals (Welsh and McClelland, 1990; Cao et al., 1998). Similarities/differences in genotypic structure play vital role in selection strategies and genetic development of bread wheat (Poehlman, 1987; Ashraf, 1994). ISSR is another DNA-based markers that have been used widespread in breeding programs, where cultivar identification, genetic mapping, genetic diversity, evolution and molecular studies are successfully made (Yang et al., 1996; Karaca and Izbirak, 2008). ISSR analysis reveals distribution of SSR in bread wheat by amplifying DNA sequences among SSRs (Chowdhury et al., 2008). This method also creates opportunity to show certain polymorphism and fingerprint structure determining genetic differences in wheat varieties genotypes (Chowdhury et al., 2008; Sofalian et al., 2009; El-Assal and Gaber, 2012). This study is aimed to determine genetic distance between some wheat varieties based on RAPD and ISSR analyses. This study could also be helpful in future for genomic mapping studies to lead development of wheat cultivars 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 wheat varieties 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 varieties then genomic DNA extracted was subjected to PCR amplification using RAPD and ISSR markers. Twelve bread wheat varieties were used and information of them including crosses was given Table 1.

RAPD and ISSR techniques, used to determine genetic distances between varieties 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 extraction 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 thermal cycler (Eppendorf Company) was DNA amplification. Five primers were chosen for ISSR analyses of genetic diversity, based on band reproducibly (Table 1).

Table 1. Pedigrees of twelve bread wheat varieties.

Tablo 1. On iki ekmeklik buğday çeşidinin pedigrileri

Bread Wheat Varieties *Ekmeklik Buğday Çeşitleri*	**Pedigrees** *Pedigriler*
Bezostaja-1	LUTESCENS17/ SKOROSPELKA2
Atay-85	HYS/7C
Sultan-95	AGRI/NAC
Harmankaya	1502-W9-01
Altay-2000	ES14//YKT/BLUEBOY2
Alpu-01	ID800994.W/VEE
Sönmez-01	BEZ//BEZ/TVR/3/KREMENA/ LOV29/4/KATEA-1
Müfitbey	NGDA146/4/YMH/TOB//MCD/ 3/LIRA/5/F130L1.12
Nacibey	F900K/3/EGL//BUC/PVN
Es-26	LLKOFEN/GEREK79*4
Yunus	SG-S1915/FANDANGO
Soyer	ATAY85/GALVEZ87

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 $MgCl_2$ 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.

Results and Conclusion

RAPD and ISSR analyses are DNA-based markers, create large enough polymorphism and fingerprint features for evaluating genetic diversity, 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. 17 of the 45 initial primers produced clear and reproducible polymorphic bands among the 12 wheat genotypes.

The 17 random primers generated a total of 103 RAPD bands and among them 97 were polymorphic (94.17%). The percentage of polymorphic bands produced by each primer ranged from 80% to 100%. The primer OPY-13 gave the highest number of RAPD bands (10), while the OPBB- 03 primers yielded the lowest number of bands (3). The band size was between 250 and 3000 bp for the primers used. Banding patterns of the 12 wheat genotypes using the primer OPY-13 are illustrated in Figure 1.

UPGMA clustering for 12 wheat genotypes showing genetic distances based on RAPD results was given in Figure 2.

Table 2. Details of banding pattern revealed through RAPD and ISSR primers(R:A,G;Y:C, T).
Tablo 2.RAPD ve ISSR primerleri ile ortaya çıkan bantların detayları (R:A,G;Y:C, T).

Markers *Markörler*	Primer/primer combination *Primer/Primer kombinasyonları*	Sequence (5'–3') *Sekans (5'–3')*	Length of amplified bands *Güçlendirilmiş bantların uzunluğu*	No of bands *Bant numaraları*	No polymorphic bands *Polimorfik olmayan bantlar*	Polymorphism ratio (%) *Polimorfizm oranı (%)*
RAPD	A-1	AGTCAGCCAC	500– 1500	5	5	100
	B-20	GGACCCTTAC	600–2000	6	6	100
	C-10	TGTCTGGGTC	400–2200	5	5	100
	OPBA-03	GTGCGAGAAC	250– 2500	8	8	100
	OPBB- 03	TCACGTGGCT	600– 1500	3	3	100
	OPA-01	CAGGCCCTTC	500– 2700	6	5	83.3
	OPA- 4	AATCGGGCTG	400– 2500	5	5	100
	OPA-13	CAGCACCCAC	750– 2300	6	6	100
	OPB- 03	CATCCCCCTG	500– 2000	6	5	83.3
	OPH- 16	TCTCAGCTGG	600– 2800	4	4	100
	OPL-09	TGCGAGAGTC	400– 2500	7	7	100
	OPL-15	AAGAGAGGGG	500– 2700	8	8	100
	OPY- 7	AGAGCCGTCA	750– 2000	6	5	83.3
	OPY-13	GGGTCTCGGT	500– 3000	10	8	80
	OPW– 8	GACTGCCTCT	400– 3000	7	6	85.7
	OPW– 17	GTCCTGGGTT	750– 2500	5	5	100
	OPW-20	TGTGGCAGCA	400– 2800	6	6	100
	Total		250– 3000	103	97	94.17
ISSR	UBC810	(GA8)T	500–2500	12	11	91.66
	UBC842	(GA)8YG	300–2000	9	9	100
	UBC868	(GAA)	400–1800	8	8	100
	SBS812	(AC)8G	300–3000	10	9	90
	SBS826	(GA)8T	500–2800	6	6	100
	Total		300–3000	45	43	95.5

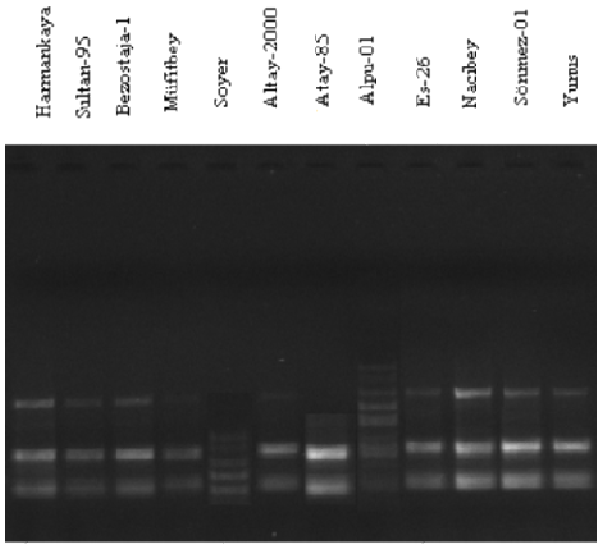

Figure 1. Result of gel electrophoresis of PCR products obtained by using (OPY-13) RAPD primer.
Şekil 1. (OPY-13) RAPD primeri kullanılarak elde edilen PCR ürünlerinin jel elektroforezi.

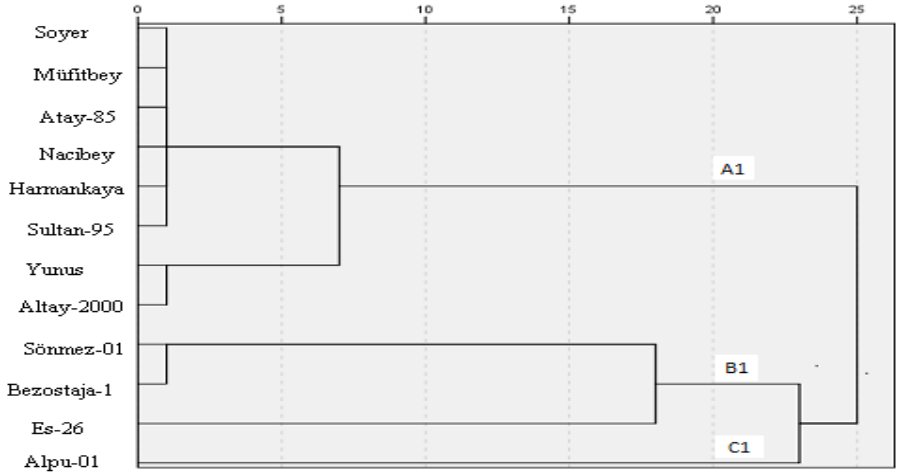

Figure 2. UPGMA clustering for 12 wheat genotypes based on RAPD markers.
Şekil 2. RAPD markörlerine dayalı 12 buğday genotiplerinin UPGMA kümeleme analizi.

The dendrogram resulted from the RAPD markers grouped the cultivars into three major clusters. Cluster A1 divided into 2 subclusters: Soyer, Müfitbey, Atay-85, Nacibey, Harmankaya, and Sultan-95 formed 1 sub-cluster, and Yunus and Altay-2000 formed the other sub-cluster. Cluster B1 divided into 2 subclusters: Sönmez and Bezostaja-1 formed one subcluster, and Es-26 another subclusters. Cluster C1 consists of Alpu-01 (Fig. 2). The similarity matrix showed that the lowest genetic similarity (0.167) was between Soyer and Alpu-01. In particular, Nacibey and Harmankaya revealed the highest genetic similarity (0.924).

ISSR analysis

In this study, five primers that showed clear and reproducible bands were obtained through screening a total of 20 primers. These 5 primers were then used to analyze genetic diversities of the 12 wheat genotype. The results of ISSR analysis are provided in Table 2. A total of 45 fragments ranging from 300 to 3000 bp were amplified with an average of 9 bands per primer, of which 44 (95.5%) were polymorphic. Each primer generated 6–12 bands and the percentage of polymorphic bands produced by each primer ranged from 90% to 100%. UPGMA clustering for 12 wheat genotypes showing genetic distances based on ISSR results was given in Figure 3.

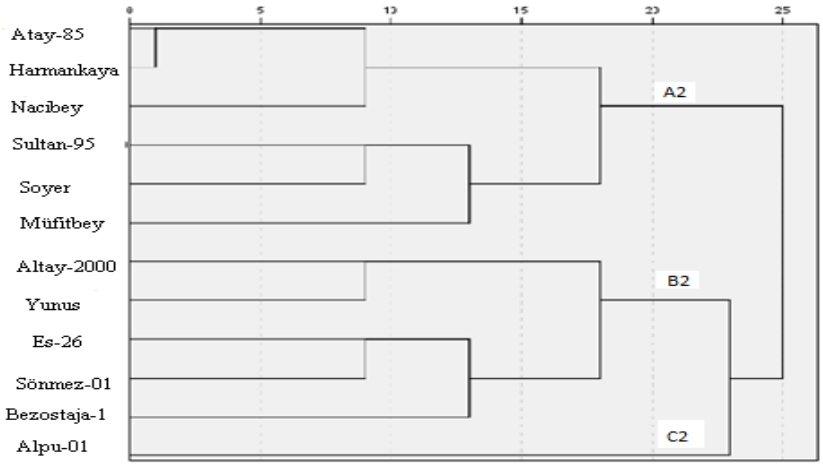

Figure 3. UPGMA clustering for 12 wheat genotypes based on ISSR markers.
Şekil 3. ISSR markörlerine dayalı 12 buğday genotiplerinin UPGMA kümeleme analizi.

The dendrogram resulted from the ISSR markers grouped the cultivars into three major clusters. Cluster A2 consists of Atay-85, Harmankaya, Nacibey, Sultan-95, Soyer, Müfitbey. Cluster B2 divided into two subclusters. Yunus and Altay-2000 formed one subcluster and Es-26, Sönmez-01, Bezostaja-1 another subclusters. Cluster C2 consists of Alpu-01 (Fig. 3). The similarity matrix showed that the lowest genetic similarity (0.199) was between Atay-85 and Alpu-01. In particular, Atay-85 and Harmankaya revealed the highest genetic similarity (0.941). The UPGMA cluster was constructed using a combination of data from the RAPD and ISSR markers and UPGMA clustering for 12 wheat genotypes based on RAPD and ISSR markers was given in Figure 4.

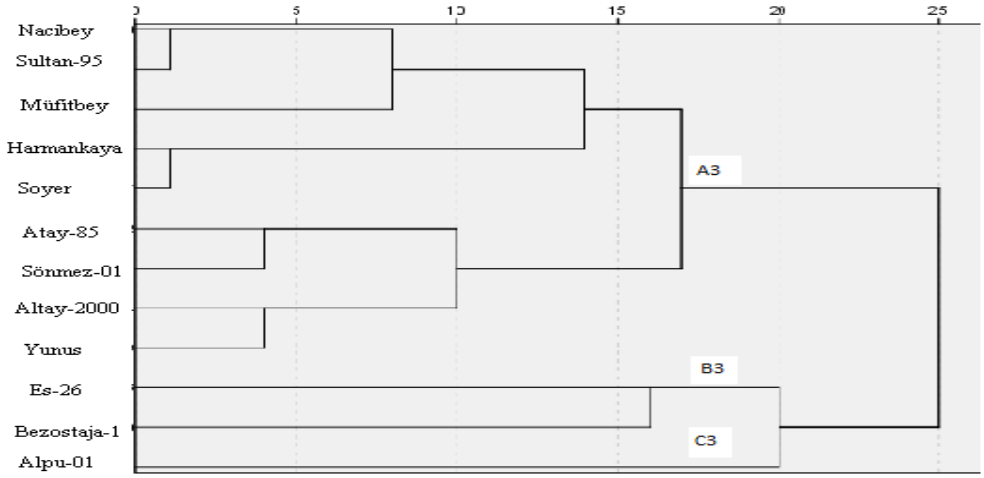

Figure 4. UPGMA clustering for 12 wheat genotypes based on RAPD and ISSR markers.
Şekil 4. RAPD ve ISSR markörlerine dayalı 12 buğday genotiplerinin UPGMA kümeleme analizi.

The 12 wheat genotypes were classified into three major groups (A3, B3 and C3). Cluster A3 divided into two subclusters. Nacibey, Sultan-95, Müfitbey, Harmankaya and Soyer formed one subcluster and Atay-85, Altay-2000, and Yunus another subclusters. Cluster B3 consists of Es-26 and Bezostaja-1. Cluster C3 had Alpu-01. The similarity matrix values varied between 0.176 and 0.953.

Bread wheat is the most cultivated, produced and consumed crop and nutritional deficit in increasing population with an incredible pace in world could be met only with the production of high-yielding varieties. This phenomenon is only accomplished by successful breeding programs having genotypic richness. Figures 2, 3 and 4 showed that Yunus and Altay-2000, Nacibey and Sultan-95, Harmankaya and Soyer were found to be close related varieties, whereas distant related varieties were Alpu-01 and Nacibey. Genetic diversities in varieties allow selection opportunity in various germplasm collections. RAPD and ISSR, time-saving molecular techniques, are coming forward and gaining importance. They could be efficiently used to evaluate genetic variation, to determine genetic diversities and improvement and development of novel varieties (Sofalian et al., 2009; Abdellatifa and AbouZeid, 2011). Moreover, genetic analyses of germplasm collection by RAPD and ISSR will assist to protect elite breeding and production materials pure, uniform and original (Nawaz et al., 2009). Studies with numerous primers in different lengths for detection of genetic polymorphisms and DNA fingerprints will increase the success.

References

Abdellatifa, K.F., Abou Zeib, H.F. 2011. Assessment of genetic diversity of Mediterranean bread wheat using

randomly amplified polymorphic DNA (RAPD) markers. Journal of Genetic Engineering and Biotechnology 9: 157-163.

Anonymous. 2011. http://www.fao.org (access date:10.03.2011).

Anonymous. 2012. http://www.tuik.gov.tr (access date:11.04.2012.

Ashraf, M. 1994. Breeding for salinity tolerance in plants. Critical Reviews in Plant Sciences 13: 17-42.

Asif, M., Rahman, M., Zafar, Y. 2005. DNA fingerprinting studies of some wheat (*Triticum aestivum L.*) genotypes using random amplified polymorphic DNA (RAPD) analysis. Pakistan Journal of Botany 37 (2): 271-277.

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.

Chowdhury, R.M.V.K., Kundu, S.J.S., R.K. 2008. Applicability of ISSR markers for genetic diversity evaluation in Indian bread wheat genotypes of known origin. Environment and Ecology 26: 126-131.

Deshmukh., R., Tomar, N.S., Tripathi, N., Tiwari, S. 2012. Identification of RAPD and ISSR markers for drought tolerance in wheat (*Triticum aestivum L.*). Physiology Molecular Biology Plants 18 (1): 101–104.

El-Assal, S.E.D., Gaber, A. 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. American Journal of Applied Sciences 9: 724-735.

Evans, L.T. Fischer, R.A. 1999. Yield potential: Its definition, measurement, and significance. Crop Science 39: 1544-1551.

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

Kang, M.S. 1990. Genotype by Environment Interaction in Plant Breeding. Department of Agronomy, Louisiana State University, Baton Rouge, Louisiana.

Karaca, M., Izbirak, A. 2008. Comparative analysis of genetic diversity in Turkish durum wheat cultivars using RAPD and ISSR markers. Journal of Food Agriculture and Environment 6: 219-225.

Karp, A., Kresovich, S., Bhat, K.V., Ayad, W.G., Hodgkin, T. 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., Rasheed, M.H. Razzaq, A. 1996. Row spacing study on two wheat variety under rainfed conditions. Sarhad Journal of Agriculture 12(1): 31-36.

Mohammed, M.I. 2009. Genotype x environment ınteraction in bread wheat in Northern Sudan using AMMI analysis. American-Eurasian Journal of Sustainable Agriculture 6(4): 427-433.

Mohapatra, T., Krishanpal, S.S., Singh, S.C., Swain, R., Sharma, K., Singh, N.K. 2003. STMS based DNA fingerprints of the new plant type wheat lines. Current Science 84(8): 1125-1129.

Mukhtar, M.S., Rahman, M., Zafar, Y. 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.

Naghavi, M.R., Mardi, M., Ramshini, H.A., Fazelinasab, B. 2004. Comparative analyses of the genetic diversity among bread wheat genotypes based on RAPD and SSR markers. Iranian Journal of Biotechnology 2(3): 195-202.

Nawaz, M., Hussain, S.A., Ullah, I., Younus, M., Iqbal, M.Z., Rana, Z.M. 2009. Estimation of genetic diversity in wheat using DNA markers. American-Eurasian Journal of

Sustainable Agriculture 3(3): 507-511.

Nei, M., Li, W.H. 1979. Mathematical model for studying genetic variations in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76: 5269-5273.

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

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. Allerd, R.W. 1984. Ribosomal spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and dynamics. Proceedings of the National Academy of Sciences 81: 8014–8019

Shashikala, S.K. 2006. Analysis of genetic diversity in wheat. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, India.,

Sofalian, O., Chaparzadeh, N., Dolati, M. 2009. Genetic diversity in spring wheat landraces from northwest of Iran assessed by ISSR markers. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37: 252-256.

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

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

Yang, W., Olivera, A.C., Godwin, I., Schertz, K., Bennetzen, J.L. 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. Crop Science 36: 1669-1676.