

Full Length Research Paper

Molecular characterization of genetic diversity in some durum wheat (*Triticum durum* Desf.) in Palestine

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Random amplified polymorphic DNA (RAPD) analysis was conducted to estimate the genetic diversity and relatedness among 10 wheat cultivars and landraces. Five out of 70 decamer random primers showed distinctly polymorphic bands when used to amplify genomic DNA. The primers yielded a total of 157 RAPD markers of which 150 were considered as polymorphic. Each primer produced 18 to 37 polymorphic bands with an average of 30 markers per primer. DNA fragments size ranged from 350 to 1910 bp. The mean similarity indices ranged from 0.05 to 0.68 with an average of 0.29. The unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on Jaccard genetic similarity index, grouped the 10 landraces and cultivars into four main clusters. Despite the clustering of most landraces in the same cluster, high genetic variation was observed within cluster-I in which the genetic distance ranged from 73% between Hiteyah8 and White Dibeyah to 32% between Black Hiteyah and Yellow Hiteyah. The result of this study confirms high genetic diversity within these cultivars and landraces, and therefore, underlined the significant values of these cultivars and landraces as gene pool for durum wheat genetic improvement. Thus, RAPD offer a potentially simple, rapid and reliable method to evaluate genetic variation and relatedness among ten wheat landraces and cultivars.

Key words: Random amplified polymorphic DNA (RAPD), *Triticum*, genetic variation.

INTRODUCTION

Wheat (*Triticum durum*, Desf.) is the most important cereal crops in the world that is, adapted to the Mediterranean region; it is the second most produced food among the cereal crops. Durum wheat is tetraploid and is of winter and spring types. Durum wheat is better adapted to semiarid climates than bread wheat. In Palestine, It covers an average area of 229,441 donum (Palestinian Central Bureau of Statistics, 2009), the total production of wheat in Palestine is 32000 ton (Palestinian Central Bureau of Statistics, 2009), with an average yield reaching 133 kg/donum (Palestinian Central Bureau of Statistics, 2009). Wheat wild relatives are still found under

natural habitats and field edges and landraces grown under traditional farming systems (Isaac and Gasteyer, 1995).

Morphological variation exists among these cultivars and landraces. Evaluation based on morphological variability is time consuming and unreliable as it is affected by environmental factor.

Molecular markers have been used thoroughly to study the genetics and genome organization of wheat (Akfirat-Senturk et al., 2010; Ercan et al., 2010; Karakas et al., 2010). It have been widely used in genetic analysis, breeding studies and assessments of genetic diversity and the relationship between cultivated species and their wild relatives, because they have numerous advantages as compared to morphological markers, including high polymorphism and independence on effects related to environmental conditions and the physiological stage of the plant (Bertini et al., 2006).

Several DNA based markers systems were introduced

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for genome mapping. Polymerase chain reactions (PCR) have become a basic and essential tool for detecting genetic variation and elucidating unknown DNA sequences (Newton and Graham, 1994). Microsatellites (simple sequence repeats, SSRs) (Nawaz et al., 2009), restriction fragment length polymorphism (PCR-RFLP) (Carvalho et al., 2009, 2011), amplified fragment length polymorphism (AFLP) (Gulbitti-Onarici et al., 2007), selective amplification of microsatellite polymorphic loci (SAMPL) (Altintas et al., 2008), expressed sequence tag (EST)-derived contigs and singletons (Karakas et al., 2010) have been widely used to characterize genetic diversity in wheat accessions, and random amplified polymorphic DNA (RAPD) (Asif et al., 2005).

RAPD has been used widely because it requires no DNA probe and no sequence information for the design of specific primers. It also allow fast and effective approaches for detecting polymorphism at the DNA level (Sivolap-Yu et al., 1997; Zvingila et al., 1998), assessment of genetic diversity and relatedness in accessions, cultivars and species (Taghian et al., 2003; Abdel-El-Haleem et al., 2009), and study of the phylogenetic relationships (Goriunova et al., 2004; Cenkci et al., 2008). It was well-known that genetic diversity in crop species is a fundamental tool in hybrid wheat breeding programs. It is therefore, useful, for breeders to know the genetic background of the breeding materials and varieties.

The goal of the study was to estimate genetic variation and relatedness among durum wheat landraces and cultivars. To our knowledge, this was the first preliminary attempt to study the molecular analysis of wheat cultivars in Palestine.

MATERIALS AND METHODS

Seed of six landraces, three commercial cultivars, and one introduced by International Center for Agricultural Research in the Dry Areas (ICARDA) of durum wheat were collected from a farmer in cooperation with the United Nations Development Programme (UNDP) and Ministry of Agriculture.

DNA extraction

Genomic DNA was extracted from the 15-days-old-seedlings germinated in the growth chamber under totally dark condition maintained at 21°C. A Promega Wizard genomic DNA purification kit was used according to instructions provided by the manufacturer. Genomic DNA quality and relative quantity were examined using 0.7% agarose gel electrophoresis detected under UV-light and photographed using a Polaroid camera with black and white 667 film. The DNA concentration was determined by spectrophotometry using a GeneQuant II, RNA/DNA calculator (Pharmacia Biotech).

RAPD analysis

The standard RAPD protocol recommended by Williams et al. (1990) was performed. A total reaction volume of 25 µl contained

2.5 µl of 10x already prepared PCR buffer; [100 mM Tris-Cl, pH 8.3, 500 mM KCl, 15mM MgCl₂, 0.01% gelatin (from Promega, catalog # M2661, Madison)]. 2.5 µl of dNTPs stock contained [2 mM of 2'-deoxyadenosine 5'-triphosphate (dATP), 2 mM of 2'-deoxycytidine 5'-triphosphate (dCTP), 2 mM of 2'-deoxyguanosine 5'-triphosphate (dGTP) and 2 mM of 2'-deoxythymidine 5'-triphosphate (dTTP) from Promega, catalog # U1330]. Five picomoles of a single 10-base primer (Operon Technologies, Inc. Alameda, USA) (primers are supplied as 0.5 OD that are resuspended in 1 ml water and used as 1 µl per reaction), 0.2 µl of (5 u/µl) *Taq* DNA polymerase (Promega, catalog # M2661) 1 µl (75 to 100 ng) of genomic DNA template, double distilled and sterile water was added to make up the final volume of 25 µl.

DNA amplification was carried out with the MJ Research model PTC-100 DNA thermocycler. The PCR program was set as: two minutes at 95°C (initial denaturing step), 40 cycles consisting of: 1 min at 94°C (denaturing), 1 min at 36°C (annealing) and 2 min at 72°C (extension, ramp time, 2 min, at 0.3°C/s), and two minutes and thirty second at 72°C (final extension step). After amplification, the tubes were stored at 4°C overnight for further use in gel electrophoresis. A total of 70 random primers were evaluated for their ability to prime PCR amplification of 10 Palestinian wheat landraces and cultivars. Amplified products were separated by 1% (w/v) agarose gels in 1X Tris-acetate buffer, stained with ethidium bromide, and visualized under UV-light.

Statistical analysis

RAPD fragments were scored as either present (1) or absent (0). The Jaccard's coefficient (Jaccard et al., 1908) was used to calculate the similarity coefficients between each pair of genotypes for all polymorphic loci using the following formula: $S_{ij} = a/(a + b + c)$, where a is the number of common bands (1, 1); b is the number of bands present in the first accession and absent in the second (1, 0); and c is the number of bands absent in the first accession and present in the second (0, 1). The matrix of genetic distances was analyzed using unweighted pair group method using arithmetic averages (UPGMA) under SPSS version 10 to cluster the entries.

RESULTS AND DISCUSSION

DNA amplification and variety identification

The genetic evaluation indicated that out of the 70 primer, 27 (37%) showed amplified fragments with mean of three fragments per each primer and 43 (62%) did not amplify detectable products (data not shown). The ones that produced unambiguous and qualitative bands in repeatable patterns when tested four to five times with the same cultivar were selected, therefore only five primers: OPA-13, OPN-14, OPN-16, OPN-18 and OPT-1 gave consistent reproducible polymorphisms with minimal smearing which were selected to analyze all the 10 durum wheat landraces and cultivars. The banding patterns of 10 wheat using OPA-13, OPN-14, OPN-16, OPN-18 and OPT-1 are shown in Figure 1.

The five primers yielded a total of 157 distinct band of which 150 (95.5%) were considered as polymorphic. The primers OPT1 produced maximum number of band (37), whereas, OPN16 generated the least number (18) of polymorphic band. The five primers produced 31 bands per primer and 30 polymorphic RAPD markers per primer

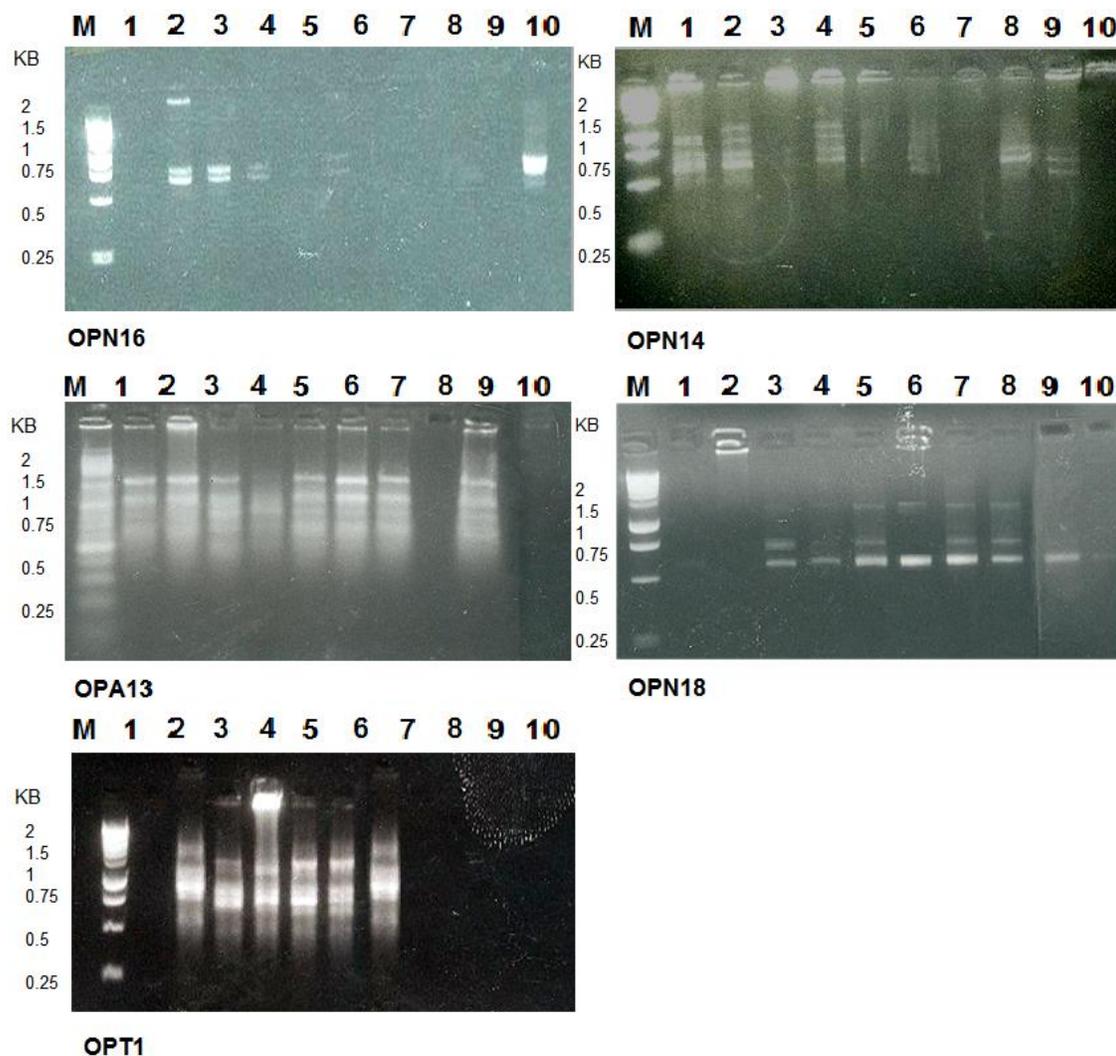


Figure 1. RAPD pattern obtained among 10 durum wheat landraces and cultivars collected from different locations in Palestine using primers OPN16, OPN14, OPA13, OPN18 and OPT1; M, molecular weight marker (1 kb DNA ladder); lane 1, White Hiteyah; lane 2, Hiteyah 8; lane 3, Black Hiteyah; lane 4, Yellow Hiteyah; lane 5, Acsad933; lane 6, 870; lane 7, Black Dibeyah; lane 8, Anbar; lane 9, White Dibeyah; lane 10, Sham 5.

Table 1. 10-Mer operon RAPD primers used in the detection of the DNA polymorphism in wheat landraces and cultivars.

Primer code	Sequence (5' to 3')	Total number of bands scored	Size range (bp)	Number of polymorphic bands
OPA-13	CAGCACCCAC	37	930-1620	35
OPN-14	TCGTGCGGGT	38	350-1910	36
OPN-16	AAGCGACCTG	19	440-1730	18
OPN-18	GGTGAGGTCA	25	610-1870	24
OPT-1	GGGCCACTCA	38	460-1510	37
Total		157		150
Average		31.4		30

(Table 1). Various numbers of primers have been used in the study of different species of the genus *Triticum* that

revealed various degrees of polymorphism. In this study, high level of polymorphism was observed as compared to

Table 2. Similarity coefficient among wheat cultivars based on Jaccard similarity index.

	HEW	HE8	HEB	HEY	AC933	V870	DEB	ANB	DEW	SH5
HEW	1.00									
HE8	0.41	1.00								
HEB	0.39	0.61	1.00							
HEY	0.35	0.50	0.68	1.00						
AC933	0.25	0.40	0.38	0.35	1.00					
V870	0.26	0.17	0.30	0.31	0.38	1.00				
DEB	0.19	0.36	0.55	0.50	0.32	0.22	1.00			
ANB	0.28	0.33	0.38	0.22	0.59	0.37	0.22	1.00		
DEW	0.15	0.27	0.38	0.33	0.17	0.13	0.38	0.18	1.00	
SH5	0.07	0.05	0.11	0.11	0.06	0.06	0.17	0.09	0.20	1.00

HEW, White Hiteyah; HE8, Hiteyah 8; HEB, Black Hiteyah; HEY, Yellow Hiteyah; AC933, Acsad933; V870, Variety 870; DEB, Black Dibeyah; ANB, Anber; DEW, White Dibeyah; SH5, Sham5.

other previous RAPD marker studies on wheat e.g. three 1998), 1.7 polymorphic band per primer in China wheat polymorphic band per primer in Pakistani (Anwar et al., 1998) cultivars (Zheng et al., 2001), 11 polymorphic band per primer in Iranian wheat cultivars (Naghavi et al., 2004) and 10.4 bands per primer in Pakistani wheat cultivars (Nawaz et al., 2009). Joshi and Nguyen (1993) used 40 primers in studying wild and cultivated wheat and revealed 88% polymorphism among all accessions.

Polymorphic band ranged in size from 350 to 1910 bp. The size out of this range was not considered in the analysis. For example, Yellow Hiteyah (4) showed DNA band outside the adapted range of DNA sizes using the OPN14. Both faint as well as strong markers were considered in scoring. The strong RAPD fragments, which were shown in some primers, that is, primers OPN18 and OPA13 could have arisen from amplifying two or more products of similar sizes. Variation in the brightness of the bands was also observed in few samples. This could be attributed to difference in template sequence, copy number and varying degrees of mismatch between the primer and the binding site (Ratnaparkhe et al., 1995).

Previous studies considered RAPD markers ranging from 200 to 1500 bp as suitable for use in further experiments as probes in southern blots or *in situ* hybridization (Williams and Ronald, 1994), while Ahmed et al. (2010) in a study of genetic diversity of 32 advanced breeding line using RAPD marker considered fragment size range from 300 to 2000 bp. The Black Dibeyah (seven) and White Dibeyah (nine) did not show any amplification using primer OPA-13, the same result was observed when primer OPN-16 was used with landraces and cultivars 10, primer OPN-18 using landraces and cultivars Hiteyah8 (two), Acsad933 (five), Anbar (eight), White Hiteyah (one) and Black Dibeyah (seven) using OPT-1 which did not show any amplification. This is expected and shows typical examples

when some reactions failed for no apparent reason.

Similarity matrix

The Jaccard (1908) genetic similarity of different landraces and cultivars pairs are shown in Table 2. A comparatively higher similarity (0.68) was observed between Black Hiteyah and Yellow Hiteyah than other cultivars and landraces combination. The lowest similarity (0.05) was found in Hiteyah8 vs. Sham5 landrace and cultivar pair. All cultivars and landraces showed an average of 0.29 genetic similarity, which could mean that the landraces and cultivars share an average of 29% of their RAPD fragments. Considering the genetic similarity values, the results indicate that the cultivars and landraces were genetically different from each other (genetic similarity value range from 0.05 to 0.68).

Cluster analysis

Dendrogram based on Jaccard genetic similarity index (1908) using UPGMA indicated a segregation of the 10 wheat landraces and cultivars into four main clusters (Figure 2), in which, five landraces Hiteyah8, Black Hiteyah, Yellow Hiteyah, Black Dibeyah, White Dibeyah constitute cluster-I and three cultivars Acsad933, Cultivars 870 and Anber grouped in cluster-III. These landraces and cultivars were grouped within the same cluster probably due to their identical genetic characteristic or might have originated from the same ancestor. The remaining clusters (II and IV) were solitary: one landrace White Hiteyah in cluster-II and Sham5 in cluster-IV. The formation of solitary cluster might be due to total isolation preventing the gene flow or intensive natural/human selection for diverse adaptive complexes. Cluster-I was also observed to divide into three sub-clusters. Black Hiteyah, Yellow Hiteyah and Hiteyah8 in

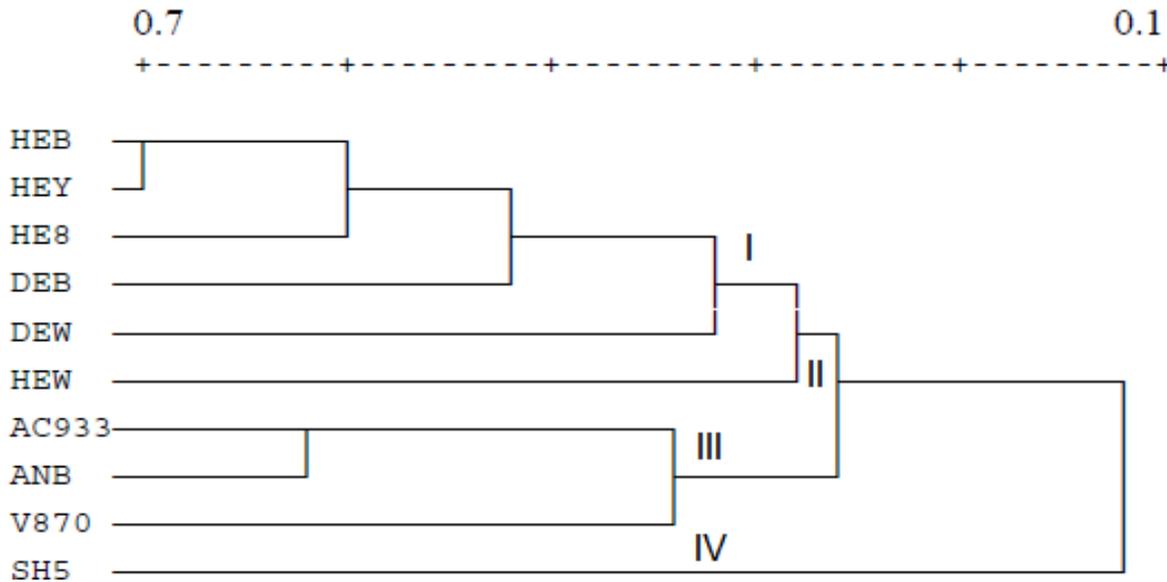


Figure 2. UPGMA dendrogram of the genetic similarity of the ten wheat cultivars based on Jaccard similarity index (Jaccard, 1908). I, Cluster-I; II, cluster-II; III, cluster-III; IV, cluster-IV; HEW, White Hiteyah; HE8, Hiteyah 8; HEB, Black Hiteyah; HEY, Yellow Hiteyah; AC933, Acsad933; V870, Variety 870; DEB, Black Dibeyah; ANB, Anber; DEW, White Dibeyah; SH5, Sham5.

sub-cluster-I with high genetic similarity ranged from 50% between Yellow Hiteyah and Hiteyah8 to 68% Black Hiteyah and Yellow Hiteyah; this might be because they have the same ancestor. Yellow Hiteyah and Black Hiteyah again together formed sub-sub-cluster-I of sub-cluster-I with the highest similarity index of 0.68 within cluster-I. Therefore, the genetic characters might be identical. Black Dibeyah in sub-cluster-II and White Dibeyah in sub-cluster-III had 38% similarity index between the two sub-clusters. The cluster-III was also further divided; Acsad933 and Anber in sub-cluster-I with 59% similarity index and 870 in sub-cluster-II with the genetic similarity of 37% between Anber and 870 and 38% between Acsad933 and 870. However, the dissimilarity within clusters-I was 73%. This means that genetic variation among sub-cluster was low. This indicates that the relatedness among landraces and cultivars within sub-cluster is high which can be due to less genetic variation among the landraces and cultivars and may originate from the same origin, or may be due to the adaptability of these landraces and cultivars to the same environment.

In conclusion, RAPD markers have been proven to be powerful tools for molecular genetic analysis of wheat cultivars for plant breeding program to assess available genetic diversity. This study indicates high genetic variability in the collected landraces and cultivars; Sham5 and Black Hiteyah cultivar and landrace showed the highest genetic variation and the lowest genetic variation, respectively, which could be used as germplasm for future cross breeding programs with the goal of improving wheat cultivars.

REFERENCES

- Ahmed MF, Iqbal M, Masood MS, Rabbani MA, Munir M (2010). Assessment of genetic diversity among Pakistani wheat (*Triticum aestivum* L.) advanced breeding lines using RAPD and SDS-PAGE. *Electronic J. Biotechnol.* 13:1-10.
- Altintas S, Toklu F, Kafkas S, Kilian B, Brandolini A, Ozkan H (2008). Estimating Genetic Diversity in Durum and Bread Wheat Cultivars from Turkey using AFLP and SAMPL Markers. *Plant Breed.* 127:9-14.
- Anwar R, Khan MA, Masood S, Tahir M, Jaradat AA (1998). Diversity in Pakistani wheat germplasm as revealed by RAPD analysis *Triticaceae* III. *Proceedings of the Third International Triticaceae Symposium, Aleppo, Syria.* pp. 171-174.
- Asif M, Rehman M, Zafer Y (2005). DNA fingerprinting studies of some wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphic DNA (RAPD) analysis. *Pak. J. Bot.* 37:271-277.
- Bertini CHCM, Schuster I, Sedyiama T, Barros EG, Moreira MA (2006). Characterization and genetic diversity analysis of cotton cultivar using microsatellite. *Genet Mol Biol.* 29:321-329.
- Carvalho A, Guedes-Pinto H, Lima-Brito J (2009). Genetic variability assessed by ITS PCR-RFLP markers in old Portuguese bread wheat. *J. Genet.* 88:363-367.
- Carvalho A, Guedes-Pinto H, Lima-Brito J (2011). Physical localization of NORs and ITS length variants in old Portuguese durum wheat cultivars. *J. Genet.* 90:95-101.
- Caneci S, Yildiz M, Konuk M, Eren Y (2008). RAPD analyses of some wild *Triticum* L. and *Aegilops* L. species and wheat cultivars in Turkey. *Acta Biol. Cracovien. Bot.* 50(1):35-42.
- Ercan S, Ertugrul F, Aydin Y, Akfirat FS, Hasancebi S, Akan K, Mert Z, Bolat N, Yorgancilar O, Altinkut-Uncuoglu A (2010). An EST-SSR marker linked with yellow rust resistance in wheat. *Biol. Plantarum* 54:691-696.
- Goriunova SV, Kochieva EZ, Chikida NN, Pukhalskii VA (2004). Phylogenetic relationships and intraspecific variation of D-genome *Aegilops* L. as revealed by RAPD analysis. *Genetika* 40(5):642-651.
- Gulbitti-Onarici S, Sancak C, Sumer S, Ozcan S (2009). Phylogenetic relationships of some wild wheat species based on the internal transcribed spacer sequences of nrDNA. *Curr. Sci.* 96:794-800.
- Gulbitti-Onarici S, Sumer S, Ozcan S (2007). Phylogenetic relationships

- among some wild wheat species revealed by AFLP markers. *Bot J Linn Soc.* 153:67-72.
- Isaac J, Gasteyer S (1995). The Issue of Biodiversity in Palestine. Applied Research Institute-Jerusalem, Palestine.
- Jaccard P (1908). New researches on the floral distribution. *Bull. Soc. Vaudoise Sci. Natl.* 44:223-270.
- Joshi CP, Nguyen HT (1993). RAPD (random amplified polymorphic DNA) analysis based intervarietal genetic relationships among hexaploid wheats *Plant Sci.* 93:95-103.
- Karakas O, Gurel F, Uncuoglu AA (2010). Exploiting a wheat EST database to assess genetic diversity. *Genet. Mol. Biol.* 33:719-730.
- Naghavi MR, Mardi M, Ramshini, HA, Fazelinasab B (2004). Comparative Analyses of the Genetic Diversity among Bread Wheat Genotypes Based on RAPD and SSR Markers. *Iran. J. Biotechnol.* 2:195-202
- Nawaz N, Hussain SA, Ullah I, Younus M, Iqbal MZ, Rana SM (2009). Estimation of Genetic Diversity in Wheat Using Dna Markers. *Am.-Eur. J. Sust. Agric.* 3(3):507-511.
- Newton CR, Graham A (1994). PCR. Bios Scientific Publisher Ltd. pp. 2-7.
- Palestinian Central Bureau of Statistics (2009). http://www.pcbs.gov.ps/Portals/_pcbs/Agriculture/tab10.htm.
- Ratnaparkhe MB, Gupta S, Venmathg MR (1995). Genetic fingerprinting of pigeonpea (*Cajanus cajan* L. Millsp.) and its wild relatives using RAPD markers. *Theor. Appl. Genet.* 91:893-898.
- Sivolap-Yu M, Kutsevich LI, Palamarchuk AI, Totsky VN, Totskii VN (1997). Molecular-genetic polymorphism of winter durum wheat determined by polymerase chain reaction with arbitrary primers. *Russ. Agric. Sci.* 1:9-13.
- Taghian AS, Abo A, Elwafa I (2003). Multivariate and RAPD analysis of spring wheat cultivars. *Anat. J. Agric. Sci.* 34(5):1-25.
- Williams CE, Ronald PC (1994). PCR template DNA isolated quickly from monocot and Dicot leaves without tissue homogenization. *Nucleic Acids Res.* 22(10):1917-1918.
- Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.
- Zheng YL, Chen YO, Wei YM, Zhou YH, Zhang ZO (2001). Esterase, gliadins and RAPD variations among Sichuan wheat cultivars. *Wheat Inform. Serv.* 92:5-8.
- Zvingila D, Kleizaitė V, Popendikyte V (1998). Analysis of RAPD and isozyme (SOD) polymorphism in cereals. *Biotechnology augalu selekcijoje*, pp. 33-37.