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Exploring Genetic Diversity in Jordanian Wheat Landraces Collected from Different Agro-ecological Regions using Random Amplified Polymorphic DNA Analysis

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ABSTRACT

Genetic diversity among twenty four wheat landraces and four Jordanian certified wheats were studied using random amplified polymorphic DNA (RAPD) technique. Highly purified DNA was obtained from wheat seeds by using Maxwell®16 DNA purification kit. A total of 981 DNA fragments (bands) were amplified, 183 of which were polymorphic. The primer OPW17, OPT03 and (OPT20, OPA15) revealed 22%, 21% and 20% polymorphism, respectively. These primers were most useful in studying genetic diversity of wheat landraces in this study. The genetic similarity between wheat landraces ranged from 0.51 to 0.02, indicating high genetic variability among tested. High similarity values were obtained between wheat landraces collected from Jerash sites, while low similarity value was found between wheat landraces collected from Ajloun locally known "Zugabiah" and between wheat landraces collected from Tafeliah site. The dendrogarm resulting from the UPGMA cluster analysis showed that the studied landraces could be divided into two main clusters from the same node. The first cluster contained five landraces that were collected from Jerash, Irbid, Ajloun, Karak and Madaba, while the second cluster contained Cham1, Dair alla, Hourani and Um qais cultivated wheat's. The dendrogram could be used for identifying the genetic variability among wheat landraces. RAPD analysis has potential to examine and assess the genetic variability among wheat varieties and landraces. © 2011 Friends Science Publishers

Key Words: Durum wheat; Genetic diversity; Jordan; Maxwell; Noorseh; Polymorphism

INTRODUCTION

Wheat is the world's most widely cultivated cereal crop (Nimbal *et al*., 2009). Durum wheat (*Triticum durum* Desf.) has a special place value in Mediterranean region and is highly appreciated for bread making and other special dishes Burghul and Freekeh. Landraces are genotypes with a mixed genetic make-up raised by natural and human selection with adaptation to harsh conditions and yield stability, which are still grown within the traditional farming systems under harsh conditions and in mountainous areas and are maintained due to their good quality. They are widely used by national, private and international breeding programs to derive genes for resistance to biotic and a biotic stresses and of good quality (Rawashdeh *et al*., 2010). Jordan constitutes a sizeable part of origin/diversity center in the Fertile Crescent, where durum landraces and primitive cultivars are being steadily replaced by improved and genetically pure cultivars (Jaradat, 1991; Rawashdeh *et al*., 2007). High variation among Jordanian durum wheat landraces was found indicating high level of diversity (Rawashdeh *et al.*, 2004; Rawashdeh *et al*., 2007).

Recently, breeding programs played a great role through in replacing landraces by highly-yield of genetically improved wheat varieties to decrease struggle the starvation worldwide and maintain food security. In addition to the chemical, morphological and cytogenetic studies, molecular markers become more common for investigation genetic diversity. Several Polymerase Chain Reaction (PCR)-based molecular markers are available and used to investigate wheat genetic diversity such as Simple sequence repeats (SSRs) (Kuleung *et al*., 2006). Inter-simple sequence repeats (ISSR) was used by Sofalian *et al*. (2008), who found that the majority of landraces from the same geographic region belonged to the same group and this confirmed that ISSR marker could be an efficient tool in cultivar identification, and Amplified Fragment Length Polymorphism (AFLP) was also used to study wheat genetic diversity (Manifesto *et al*., 2001; Hazen *et al*., 2002; Tyrka, 2002; Maher *et al*., 2005; Khalighi *et al*., 2008). Also, Dholakia *et al*. (2003) used SSRs and RAPD to analyze the kernel traits in different recombinant inbred line population in order to find additional loci controlling kernel size and shape. Their results showed that the number of markers

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associated with each trait ranged from two to nine and many of the markers showed linkage to more than one trait. PCR-RAPD markers have been shown to be an effective method for studying genetic diversity and detection of polymorphism in wheat (Aliyev *et al*., 2007; Rashed *et al*., 2008; Sawalha *et al*., 2008; Tahir, 2008; Nimbal *et al*., 2009; Rawashdeh & Allozi, 2009; Khan *et al*., 2010; Rawashdeh *et al*., 2010; Salem *et al*., 2010).

The present study was therefore undertaken to study the genetic diversity among Jordanian wheat landraces collected from six governorates as revealed by RAPD markers.

MATERIALS AND METHODS

Wheat landraces collection: The landraces materials collected from six governorates representing with a wide range of agro-climates conditions (Table I) and seeds of four cultivated wheat varieties provided to us from stores by Al-Mushaqer Research Station/National Center for Agricultural Research and Extension (NCARE) were assessed for genetic diversity. In addition to, 50 samples from different spikes of wheat landrace Noorseh (collected from Bait Yafa'a-Irbid the seeds & spike shapes shown in Fig. 1) was also used for assessing the genetic variability within this landrace.

DNA isolation from wheat landraces: Genomic DNA was isolated from the seeds of 28 wheat genotype (Table I) by using Maxwell®16 System isolation machine and its procedure (Promega) at NCARE, which was the first time that this machine was used to obtain DNA from wheat seeds. The DNA isolation followed the procedure described by (Maxwell®16 DNA purification kits (Cat. No. AS1030; www. promega.com). Two seeds per wheat landrace were manually digested on the aluminum foil then placed in the 1.5 mL tubes. Then 100 µL of XG buffer, 10 µL of 10% Triton® X-100 and 10 µL of CelluACE enzyme were added. The tubes were incubated at 50ºC for two hours with constant vortexing. Cartridge samples, including with the Maxwell®16 tissue DNA purification, kit were placed into the holder with the ridge side of the cartridge facing toward the numbered side of the rack, then the seal was removed from each cartridge. The plungers were placed into well no. 7 of each cartridge (Technical Manual Maxwell®16 DNA purification kit). The whole mix was transferred into well no. 1. The cartridge containing the samples and plungers was transferred from the cartridge preparation rack into the Maxwell®16 platform. One blue elution tube was placed for each cartridge into the elution tube slots at the front of the platform. 300 µL of elution buffer was added to each blue elution tube.

After running the machine for 45 min highly purified DNA was obtained. The elution tubes were removed from the platform-heated elution tube slots and placed them into the Magnetic elution tube rack. The eluted samples were transferred into 1.5 mL tubes and stored at -20ºC for DNA analysis. The quality of DNA was detected by running 10 µL DNA on 0.70% agarose gel prepared in 0.5 X TBE buffer.

DNA isolation from "Noorseh" wheat landrace: DNA was isolated from 21 days old leaves at the seedling stage following the procedure as described by Doyle and Doyle (1987) with minor modifications. Approximately 20 mg of fresh leaves were grounded in liquid nitrogen and mixed with 600 µL of freshly and preheated 2X CTAB solution with 0.8 g PVPP in 2 mL tubes then placed at 65ºC for 30 min. The mixture was added to 600 µL of chlorophorm/isoamyl alcohol (24:1), vortexed shortly for few seconds and then centrifuged at 13000x g for 10 min. The supernatant was placed in 2 mL tubes with 600 mL isopropanol and then shacked until the thread of DNA appeared, then centrifuged for 10 min at 13000x g. The solution is poured in tubes and left to dry, then 600 µL of cold 70% ethanol was added to the solution and placed in the refrigerator at –20ºC overnight. Next day, ethanol was poured in the dried tubes and 150 µL of TE was added and the whole mixture was placed at 65ºC for 60 min. 4 µL of RNAase (10 mg/mL) was added into each tube and left for 60 min at 37ºC. Finally the DNA quantity was measured using S2100 UV/VIS DIODE-Array-Spectrophotometer, machine Version 1.7. The quality of DNA was checked by running 10 µL DNA on 0.70% agarose gel prepared in 0.5X TBE buffer.

PCR amplification: PCR reaction was performed as described by Williams *et al*. (1990) with 10-mer ologonucleotides synthesized by Operon technologies (Almeda, Calif.). The final volume of 25 µL contained 10 x buffer with MgCl2, 20 ng of total genomic DNA, 0.25 mM dNTPs (Promega), 100 µM of primers, 1.5 mM MgCl2 and 1 U of *Taq* polymerase. Amplification was carried out in thermocycler (MJ Research, USA, Model PCT-200), with one cycle of 1 min at 94°C followed by 44 cycles, each of which consisted of a denaturation step for 1 min at 94°C, followed by an annealing step for 1min at 36°C and an extension step for 2 min at 72°C, ending with a further extension step for 5 min at 72°C. After the final cycle the samples were cooled at 4°C. Samples of 10 µL RAPD-PCR product were analyzed by electrophoresis on 1.4% a garose gel and visualized after staining with ethidium bromide. Forty-four 10-mer primers were screened (Table II) and 10 primers showing polymorphism were used for studying genetic diversity among wheat landraces (Table III). Five polymorphic primers were used to assess the genetic variability within "Noorseh" wheat landrace (Table IV).

Data analysis: RAPD bands were manually scored as present (1) or absent (0) for estimating the similarity among all tested samples. The matrix of similarity (Jaccard) and similarity of coefficients (Nei & Li, 1979) were calculated and the dendrogram was obtained by clustering according to the Un-weighted Pair-Group Method with Arithmetic averages (UPGMA) using SPSS (V.11.0), software. Polymorphism percentage was estimated by dividing the number of polymorphic bands over the total number of bands.

RESULTS

Wheat landraces found in a wide range of altitudes from 40 m below sea level (Wadi Sair–Wadi Shatta) up to 1569 m above sea level (Tafeliah-Mine site) (Table I). Noorseh wheat landrace was collected from two elevations, one from 745 m in Hussban and the other from Bait Yafa´a–Irbid (Table I). Qattmah landrace collected from Al-Karak 2 (Table I) and from Wadi Sair 4 were not grouped, also Noorseh collected from Bait Yafa´a–Irbid and Hussban had the same characteristics of the spike and seed features but they did not cluster in the same group. Highly purified DNA was obtained from wheat seeds by using Maxwell®16 DNA purification kit. Forty four RAPD primers were tested, but only 10 showed reproducible fragments with easily recordable bands. Total number of bands, number of polymorphic bands and percent of polymorphism and maximum/minimum number of bands per primer are shown in Table III and Table IV. A total of 981 RAPD fragments were consistently recognized and 183 of them were polymorphic wheat landraces, while 757 RAPD fragments were obtained by using 5 high polymorphic primers for testing the genetic variability within Noorseh landrace, among, which 102 were polymorphic (Table IV). OPA15, OPC03, OPTO3, OPT05, OPT16, OPT20, OPW04, OPW17, OPZ08 and OPZ20 primers were used for differentiation among landraces studied. Meanwhile, OPC03, OPC09, OPZ06, OPZ08 and OPZ18 were used in testing variability within Noorseh landrace (Table IV). An example of DNA banding patterns among wheat landraces and Noorseh landrace is given in Fig. 2 and 3.

Levels of similarity between wheat landraces ranged from 0.51 to 0.02 (Table V). The highest average similarity index value of (0.51) was observed between two samples collected from Jerash. Sample of wheat landrace collected from Tafeliah had a 0.05 similarity with Noorseh landrace. For Noorseh landrace, the similarity values ranged from 0.85 to 0.00 (No similarity), with the highest similarity values recorded between two samples No. 29 and 30 (0.85), 31 and 33 (0.72), 26 and 32 (0.71), 20 and 22 (0.55), 8 and 9 (0.63), 12 and 13 (0.58), 6 and 16 (0.72) 37 and 38 (0.72) and 48 and 49 (0.75) (Table VI).

The dendrogram can be sub-divided arbitrarily into two main clusters. The first cluster included the wheat landraces collected from the following regions; Jerash 1 and Jerash 2, Irbid 1 and Irbid 4, Ajloun 1 and Ajloun 2, Karak 1, Karak 2, Ajloun1, Karak 5, Madaba 1, Karak 3, Karak 4 and Madaba 2 (Fig. 4). The second cluster contained the following wheat landraces: Wadi Sair 6, Wadi Sair 7, Naoor, Wdai Sair 4, Wadi Sair 1, Wadi Sair 3, Madaba 3, Tafeliah, also UmQais, Dair alla, Hourani and Cham1 (Sham 1) cultivated varieties. The dendrogram obtained for Noorseh wheat landrace can be subdivided into three main clusters; first cluster included samples No. 3 to 38, the second contained sample No. 39 to 50 and the third included two samples; 1 and 2 (Fig. 5).

Fig. 1: Spikes and seeds shape of Jordan "Noorseh" wheat landrace

Fig. 2: An example of RAPD patterns obtained from primer OPW17 on 14 wheat landraces collected from different regions in Jordan Lane 1: Jerash1; lane 2: Jerash 2; lane 3: Irbid 1; lane 4: Irbid 2; lane 5: Ajloun 1; lane 6: Ajloun 2; lane 7: Ajloun1, lane 8 to 12, Karak1, 2, 3, 4, and 5 respectively; Lane 13: Madaba1; lane 14: Madaba 12 (Table 1). M: 1kb ladder

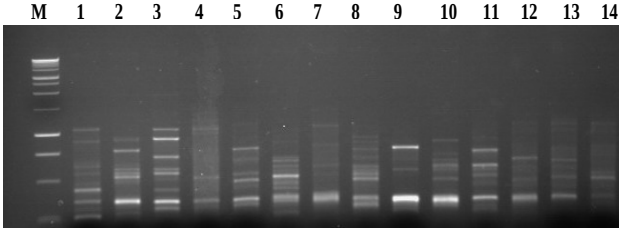
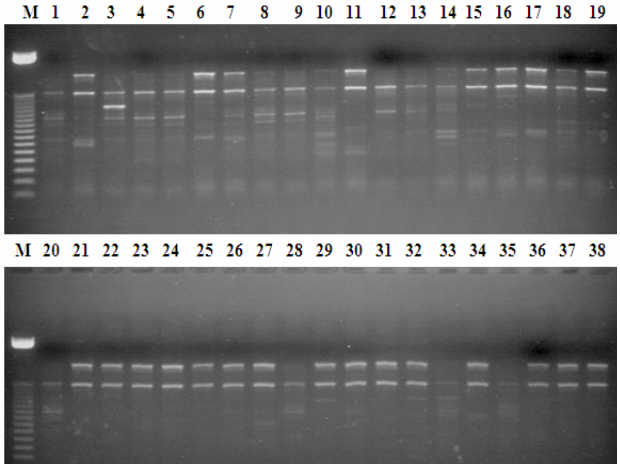


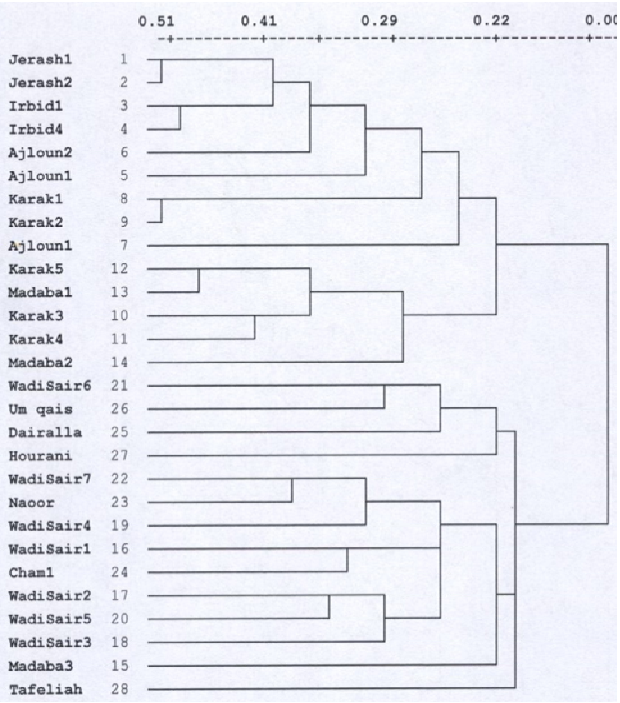
Fig. 3: An example of RAPD patterns obtained from primer OPC03 on 38 wheat Noorseh wheat landrace collected from Irbid region in Jordan, M: 50 bp ladder



DISCUSSION

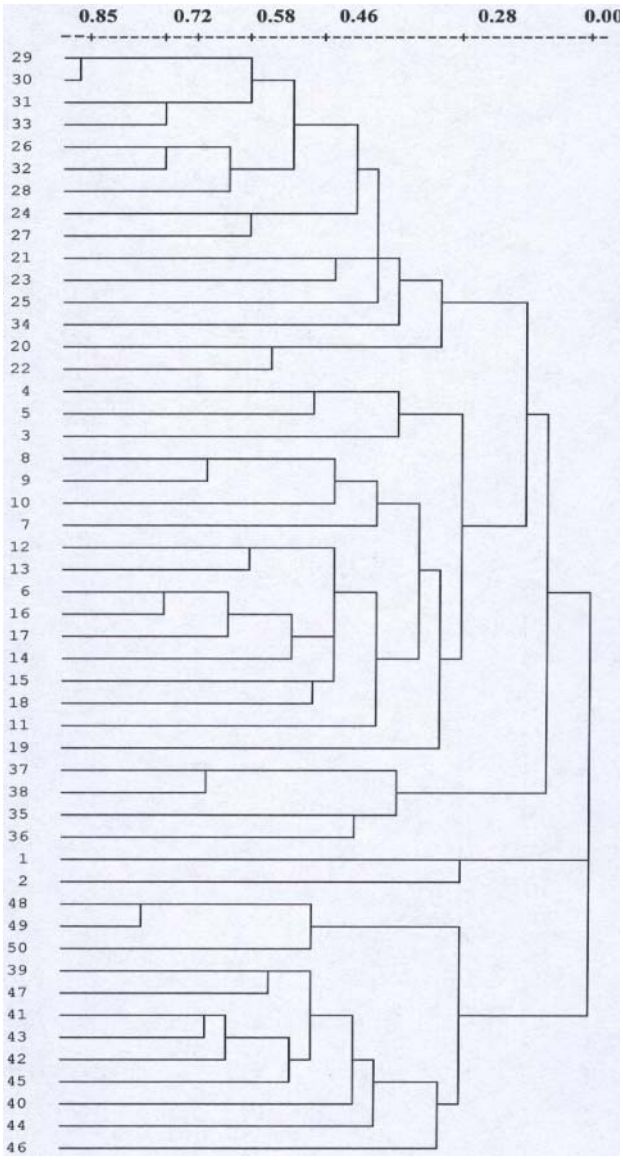
Estimation of genetic diversity among and within crops is crucial in breeding and conservation of genetic resources. Wheat is an essential crop and important element

Fig. 4: A dendrogram among wheat landrace collected form six governorates in Jordan using 10 polymorphic RAPD primers based on Jaccards´ coefficient of similarity



in agro biodiversity and food security (Sawalha *et al*., 2008). Various number of RAPD primers were used in the study of genetic diversity among different wheat genotypes for example, five different primers (Sawalha *et al*., 2008), four primers used by (Aliyev *et al*., 2007) and five primers (Rashed *et al*., 2008). The results of amplification by PCR for the wheat varieties with five arbitrary RAPD primes indicated distinct differences for identification of wheat varieties (Rashed *et al*., 2008). In our investigation, out of 44 RAPD primers, 10 polymorphic primers were used for studying the genetic diversity showed feasibility for estimating the genetic diversity among wheat landraces and also five RAPD primers showed high polymorphism among samples of Noorseh wheat landraces. The results confirmed the potential of using DNA finger printing to estimate the genetic relatedness and diversity among landraces and varieties of wheat species. The screening of over 2000 Mexican landraces exhibiting considerable phenotypic diversity in yield under drought, suggested that DNA fingerprinting can be used to confirm genetic difference between landraces and checks as well as among landraces themselves (Reynolds *et al*., 2007). Genetic variability found among and within wheat landraces could be manifested by variability in grain protein concentration, which provides foundation for introgression of high protein genes into wheat genotypes. Seed storage proteins are the result of expression of genome and contain extensive genetic variation in wheat landraces (Chaparzadeh *et al*.,

Fig. 5: A dendrogram among Noorseh landrace using five polymorphic RAPD primers based on Jaccards´ coefficient of similarity



2008). The obtained results can be further used to determine the most important wheat grain quality traits mainly grain protein based on DNA markers through genetic enhancement by selection for potent genotypes (Nimbal *et al*., 2009).

Relatively high similarity values obtained between some of the wheat landraces collected from the same districts showed possible geographic linkage through natural selection and possible gene exchange, and exchange of seed among neighboring farms as in the case of landrace samples collected from Ajloun and Karak districts, which could trace back to seeds distributed during field on-farm demonstration trial (Rawashdeh *et al*., 2010). In our study, Ajloun and Karak landraces were grouped together, which is in

Table I: The wheat landraces collected from various governorates of Jordan

Region	Location	Type	Altitude	ºE	ºN
Jerash *1	Juba	*T. durum,* yellow spike	986	035.92895	32.32934
Jerash 2	Main road	Black awns ,*T. durum* **Dubiah	537	035.88944	32.26859
Irbid 2	Bait Yafa'a	*T. durum* - Noorseh	745	035.81585	32.50140
Irbid 1	Al-Husoon	*T. durum* ,white and yellowish spike	697	035.87233	32.47823
Ajloun 5	Muneef head	*T. durum*	1159	035.81447	32.37740
Ajloun 3	Nellah	*T. aestivum*	50	035.64830	32.26823
Ajloun 3	Nellah	*T. durum,***Zugabiah	50	035.64830	32.26823
Al-Karak 1	Myassar	*T. durum*	948	035.73693	31.39283
Al-Karak 2	Al-Yaroot	*T. durum,* **Qattmah	922	035.72233	31.31034
Al-Karak 3	Al-Rawthah	*T. durum,*-white Yellowish	986	035.73363	31.25019
Al-Karak 4	Al-Rawthah	*T. durum,* **Qattmah	986	035.73363	31.25019
Al-Karak 5	Al-Rawthah	*T. durum,* white spike	986	035.73363	31.25019
Hussban 1	Wadi hussban	*T. durum*- Noorseh , white spike	633	035.79416	31.82441
Madaba 2	Muragmeh	*T. durum*	711	035.78054	31.64521
Hussban 3	Wadi hussban	*T. durum*- white spike	633	035.79416	31.82441
Wadi Sair 1	Iraq-Al-Ameer	*T. durum,* black awns	427	035.75073	31.91361
Wadi Sair 2	Iraq-Al-Ameer	*T. aestivum*	427	035.75073	31.91361
Wadi Sair 3	Iraq-Al-Ameer	*T. durum,* white spike	427	035.75073	31.91361
Wadi Sair 4	Iraq-Al-Ameer	*T. durum,* white spike,**Qattmah	427	035.75073	31.91361
Wadi Sair 5	Wadi shatta	*T. durum*	40	035.73305	31.87836
Wadi Sair 6	Sawtt hunittay	*T. durum,* 50 years old	725	035.79265	31.93266
Wadi Sair 7	Sawtt hunittay	*T. durum*	725	035.79265	31.93266
Naoor	Main road	*T. durum*	742	035.78600	31.85088
***Cham1 (Sham 1)	Al-Mushaqer Research Station	*T. durum*	-	-	-
***Diar alla	Al-Mushaqer Research Station	*T. durum*	-	-	-
***Um qais	Al-Mushaqer Research Station	*T. durum*	-	-	-
***Hourani	Al-Mushaqer Research Station	*T. durum*	-	-	-
Tafeliah	Tafeliah Concrete mine-Rashadiah	*T. durum*	1569	035.62402"	30.67879

*Site number, **locally names nominated by farmers, *** Wheat varieties, WANA catalogue of crop varieties, 1998. WANA seed network publication No.18/98

Table II: Primers names and their sequences used in this study

Primer name		Sequence 5'-3'	Primer name	Sequence 5'-3'
1.	OPA09	GGGTAACGCC	23. OPC18	TGAGTGGGTG
2.	OPA10	GTGATCGCAG	24. OPC20	ACTTCGCCAC
3.	OPA13	CAGCACCCAC	25. OPD04	TCTGGTGAGG
4.	OPA15	TTCCGAACCC	26. OPD06	ACCTGAACGG
5.	OPA16	AGCCAGCGAA	27. OPD10	GGTTCACACC
6.	OPA18	AGGTGACCGT	28. OPD11	AGCGCCATTG
7.	OPA20	GTTGCGATCC	29. OPD14	CTTCCCCAAG
8.	OPB01	GTTTCGCTCC	30. OPD16	AGGGCGTAAG
9.	OPB04	GGACTGGAGT	31. OPD18	GAGAGCCAAC
10.	OPB05	TGCGCCCTTC	32. OPD20	ACCCGGTCAC
11.	OPB06	TGCTCTGCCC	33. OPT03	TCCACTCCTG
12.	OPB08	GTCCACACGG	34. OPT05	GGGTTTGGCA
13.	OPB09	TGGGGGACTC	35. OPT10	CCTTCGGAAG
14.	OPB10	CTGCTGGGAC	36. OPT12	GGGTGTGTAG
15.	OPB12	CCTTGACGCA	37. OPT15	GGATGCCACT
16.	OPB13	TTCCCCCGCT	38. OPT16	GGTGAACGCT
17.	OPB14	TCCGCTCTGG	39. OPT19	GTCCGTATGG
18.	OPB17	AGGGAACGAG	40. OPW04	CAGAAGCGGA
19.	OPB19	ACCCCCGAAG	41. OPW17	CTCCTGGGTT
20.	OPC03	GGGGGTCTTT	42. OPZ06	CCAGGAGGAC
21.	OPC09	CTCACCGTCC	43. OPZ08	CCGACAAACC
22.	OPC10	TGTCTGGGTG	44. OPZ18	AGGGTCTGTG

Table III: Total bands, number of polymorphic bands and percent of polymorphism per primer of most polymorphic RAPD primers used for wheat landraces under this study

Primer name	Total bands/primer	Number of polymorphic bands	% of polymorphism
OPA15	83	17	20
OPC03	38	6	16
OPT03	98	21	21
OPT05	165	30	18
OPT16	72	14	19
OPT20	159	32	20
OPW04	91	16	18
OPW17	147	33	22
OPZ08	72	8	11
OPZ18	56	6	11
Total bands	981	Average :18.3	Mean: 17.6

agreement with previous results found by Rawashdeh *et al*. (2010). Wheat landraces collected from Irbid, Jerash and Ajloun are grouped together. Those Governorates are located at Northern part of Jordan and more closer compared to other Governorates. The grouping might be confirmed by exchange of wheat landraces between the farmers. High similarity found within samples of Noorseh wheat landrace could be due to several reasons. Firstly, RAPD primers used might be matched at same sites on DNA sequence. Secondly seeds are generated from the same parents. Thirdly, it can be caused by gene introgression. Sham 1 and ACSAD 65 durum wheat improved cultivars were located in two different clusters, which are far from each other indicating their weak genetic relationship. This may possibly be resulted from different genetic backgrounds they derived from two breeding programs (Rawashdeh *at al*., 2010). Similar reason can be used to explain our findings that Dair alla, UmQais and Hourani cultivated wheat varieties were grouped in one sub-cluster but Cham 1 formed with a separate cluster, this could

Table IV: Total bands, number of polymorphic bands and percent of polymorphism per primer of most polymorphic RAPD primers used for Noorseh wheat landrace under this study

Primer name	Total bands/primer	Number of polymorphic bands	% of polymorphism
OPC03	145	20	14
OPC09	78	7	9
OPZ06	226	23	10
OPZ08	200	36	18
OPZ18	108	16	15
Total bands	757	Average : 20.4	Mean: 13.2

Table V: Selected highest and lowest similarity values based on similarity coefficient of the amplified bands between Jordanian wheat landraces

Sample no. (see table I)	Sample no. (see table I)	Similarity values
1	1	0.51
2	3	0.48
3	4	0.49
4	5	0.38
5	6	0.33
8	9	0.50
11	12	0.40
12	13	0.47
1	23	0.05
2	24	0.03
3	28,27	0.03
1	19,18	0.04
4	28,27	0.04
13	23	0.05
6	28	0.02

Table VI: Selected highest and lowest similarity values based on similarity coefficient of the amplified bands between samples of "Noorseh" landrace collected from Irbid site (Bait Yafa'a) in Jordan

Sample no. (see Fig. 4)	Sample no. (see Fig. 4)	Similarity values
4	5	0.50
6	16	0.72
7	9	0.50
8	9	0.63
12	13	0.58
14	16	0.62
16	17	0.68
20	22	0.55
25	29	0.69
29	30	0.85
31	33	0.72
37	38	0.72
41	43	0.65
48	49	0.75
50	5,4,3	0.00
45,44,43	4	0.00
23	44,43	0.00
30,29	45,44	0.00
9,8	50	0.03
27	42 to 46	0.03
26	50,49	0.04

be related to differences in their genetic backgrounds they derived from two separated breeding programs. The results from this study will contribute to future research aiming at improving Jordanian wheat landraces using Marker–Assisted-Selection (MAS). Yellowness color of wheat seeds is as an accumulation of caroteniods pigment in the endosperm and may be a landrace-specific trait. It is considered as a quality trait that can be used to differentiate among durum wheat landraces.

From the above results, we conclude that (1) track the genes encoding enzymes of biosynthetic carotenoid pathway; (2) determine protein contents percentages and (3) link genetic markers to specific commercial traits, which may assist in selection and plant cultivation and management strategies.

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