MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF SOME LENTIL GENOTYPES

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Abstract

Ten lentil genotypes were analyzed for their chemical composition. Crude seed protein ranged between 33.22% in FLIP 2003-57L and 23.52% in XG98-3-2. The range of total seed carbohydrate percent was 63.60% for FLIP2003-54L and 55.87% for XG 98-9-1. Iron level in the ten lentil genotypes ranged from 4.7 for Giza 9 to 20.8 for XG 98-9-1 (mg/100g). The level of zink ranged from 2.6 for FLIP 2003-54L and 4.8 mg/100g for XG 98-9-1.

Genetic diversity of the 10 lentil genotypes was studied using RAPD-DNA markers and SDS-PAGE of seed proteins. Application of 11 RAPD arbitrary primers resulted in a total of 119 bands; of which 67 bands were polymorphic (56.30%). The genetic distance matrix based on Dice Distances, revealed a considerable level of genetic diversity among genotypes. Maximum similarity was observed between ILL7163 and each of XG 98-1-1 and Giza 51 with 95.5% similarity, while Sinai 1 and Giza 9 were distantly related with 23.2% similarity. RAPDs generated a large set of markers, which can be useful as specific markers for some genotypes. Electrophoresis of total seed proteins revealed slight variation in protein profiles among lentil genotypes. Cluster analysis for the combined data of RAPD and SDS-PAGE revealed two main clusters – Cluster I with Giza 9 and Cluster II containing all the remaining genotypes. Giza 9 was found to be the most distinct in simple as well as combined analyses. The combined data also showed that an average genetic similarity of 87.9% (ranging from 80.4 to 95.4) was found among the genotypes. The highest similarity was observed between XG96-5-39 and XG 98-1-1 which were 95.4% followed by 95% between ILL7163 and each of XG 98-1-1, Giza 51 and XG 98-9-1. However, the lowest similarity was 80.4%, which observed between Giza 9 and Sinai 1. The results of this study indicate that RAPD DNA markers and seed protein profiles seem to be suitable for assessing genetic diversity among lentil genotypes for future breeding programs. The result showed one RAPD marker, which was linked to high iron at 1226 bp of primer OP-A10, which could be used as rapid marker in selection for this trait in breeding programs.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is one of the oldest and most important grain legumes in the world. It acts as a valuable meat substitute in many subsistence communities. Lentil is a highly valued annual food legume crop in the Near East area, since 8000 years ago (Cubero, 1981). More recently, the crop has been successfully introduced to the New World, where the leading producers are Argentina, Chile and

the USA. The cultivated lentils and their wild relatives are self-pollinating diploids (2n = 2x = 14). A pre-requisite for the efficient use of genetic resources in all plant-breeding programmes is important for the extent and distribution of the genetic variation available within the Genus. During the past 15 years numerous attempts have been made to examine the diversity and phylogeny of *Lens* (Sharma *et. al.* 1995). Lentil and other legumes have the third-highest level of protein, by weight, of any plant-based food after soybeans and Hemp. Also, lentil has great potential as a whole food source of Fe and Zn for people affected from nutrient deficiencies (Thavarajaht *et. al.* 2009). Deficiencies of Fe and Zn in human populations are widespread, affecting up to two billion people. Although these trace elements are required only in small amounts, they are of comparable importance nutritionally to vitamins.

Seed storage protein profiling based on SDS-PAGE can be employed for various purposes, such as varietal identification, biosystematics analysis, determination of phylogenetic relationship between different species, generating pertinent information to complement evaluation and passport data (Malviya *et. al.* 2008). SDS-PAGE can be a useful technique in cultivar identification (Abdel-Tawab *et. al.*,1993). Hamdi and Omar (2006) used SDS-PAGE seed protein technique for biological identification of five lentil cultivars.

The understanding and knowledge of genetic variation and genetic similarities present within individuals or populations are useful for the efficient use of genetic resources in breeding programs. The breeder can use the genetic similarity information to complement phenotypic information in the development of breeding populations. Patterns of genetic variation and genetic similarity have frequently been studied within and among crop species using both morphological and isozyme markers, but they have several drawbacks, such as their limited number, environmental dependence, and uneven distribution (Ertug *et. al.*, 2006).

DNA marker systems are useful tools for assessing genetic diversity levels among germplasm (Lee, 1995, Karp *et. al.*, 1996) compared with pedigree information. DNA marker-based diversity estimates better reflect actual DNA differences among lines. The PCR-based techniques such as randomly amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) can provide useful markers in studies of genome evolution, analysis of genome composition, and genome identification (Welsh *et. al.*, 1991).

MATERIALS AND METHODS

Ten lentil genotypes were used in this study, namely: Giza 9, XG 98-1-1, Giza 51, XG96-5-39, Sinai 1, FLIP 2003-57L, XG 98-9-1, FLIP2003-54L, XG98-3-2 and ILL7163.

Chemical composition:

Samples of about 50g of air dried finely ground seeds of each genotype were used for estimating seed chemical composition. Crude protein and total carbohydrates were determined according to the methods of (AOAC 2000).

Micro-elements:

Fe and Zn in lentil genotypes were determined by flame atomic absorption spectrophotometer.

SDS-Polyacrylamide gel electrophoresis:

SDS-PAGE was performed according to the method of Laemmli (1970) as modified by Studier (1973).

Molecular markers:

Genomic DNA extraction

DNeasy plant minikit (Quigen Inc., Cat.no.69104, USA) was used for DNA extraction.

RAPD-PCR analysis:

RAPD – PCR reactions were conducted using 11 arbitraty 10- mer primers with the 5′ → 3′ sequences as shown in Table (1).

The reaction conditions were optimized and mixtures were prepared (30 µl total volume) consisting of the following: dNTPs 2.4 µl, $MgCl_2$ 3.0 µl, 10 x buffer 3.0 µl, primer (10 um) 2.0 µl, Taq (5u/µl) 0.2 µl, template DNA (50 ng / µl)2.0 ul, H2O (dd) 17.4 ul. Amplification was carried out in a PTC- 200 thermal cycler (MJ Research, Watertown , USA) programmed as follows : denaturation, 94°C for 3 minutes, then for 40 cycles. Each cycle consisted of 1 minute at 94°C, 1 minute at 37°C, 2 minutes and 30 second at 72°C, followed by a final extension time of 12 minutes at 72°C and 4°C (infinitive).

Gel electrophoresis was applied according to Sambrook et al. (1989). Agarose (1.2 %) was used for resolving the PCR products. The run was performed for one hour at 80 volt in pharmacia submarine (20 x 20 cm). Bands were detected on UV–transilluminator and photographed by Gel documentation 2000, Bio- Rad.

Table 1. Random primers codes and their sequences for RAPD- PCR analysis.

Primer name	*Sequence*
OP- A01	5′ CAGGCCCTTC 3′
OP-A08	5′ GTGACGTAGG 3′
OP-A10	5′ GTGATCGCAG 3′
OP-A12	5′ TCGGCGATAG 3′
OP-B07	5′ GGTGACGCAG 3′
OP-B11	5′ GTAGACCCGT 3′
OP-B13	5′ TTCCCCCGCT 3′
OP-B14	5′ TCCGCTCTGG 3′
OP-B18	5′ CCACAGCAGT 3′
OP-B19	5′ ACCCCCGAAG 3′
OP-B20	5′ GGACCCTTAC 3′

RESULTS AND DISCUSSION

Chemical Composition

Lentil seeds of the ten lentil genotypes were analyzed for their chemical composition (Table 2). FLIP 2003-57L recorded the highest percent of crude protein, which was 33.2% followed by 30.8% for XG96-5-39, while the lowest percent was 23.5% for XG98-3-2. The percent of total carbohydrates ranged from 63.6% (FLIP2003-54L) to 55.9% (XG 98-9-1). These results were in agreement with those of Khan *et. al.* (1987) and El-Nahry et al. (1980). Concentration of chemical composition in lentil mentioned in this paper mostly agreed with those reported earlier. Disagreement found in some genotypes could be due to genetic background and agroclimatic conditions in which the genotypes were grown.

Micro-elements:

The detected levels of Fe and Zn in the ten lentil genotypes ranged from 4.7 to 20.8 mg/100g and from 2.6 to 4.8 mg/100g, respectively, which agreed with those of Khan *et. al.* (1987). The highest values of Fe and Zn were found in XG 98-9-1, while the lowest values of Fe and Zn were observed in Giza 9 and FLIP2003-54L, respectively. Mestek *et. al.* (2002) reported that Fe and Zn contents in lentil were 8.15 and 4.21 mg per 100 g sample, respectively. In another study, Erdogan et al. (2006) found that Fe and Zn contents in lentil have been reported as 8.24 and 2.46, respectively. Some genotypes in our study were superior in these elements and can be used as a good food source of Fe and Zn.

Table 2. Chemical characteristics and micro-element of the ten lentil genotypes.

Genotypes	% Crude protein	% Total carbohydrates	Fe (mg/100g)	Zn (mg/100g)
Giza 9	28.4	59.9	4.7	3.2
XG 98-1-1	27.8	60.1	16.3	3.5
Giza 51	28.6	56.7	11.2	4.1
XG96-5-39	30.8	56.9	7.5	3.4
Sinai 1	27.3	60.1	15.8	3.5
FLIP 2003-57L	33.2	56.0	10.1	4.0
XG 98-9-1	24.3	55.9	20.8	4.8
FLIP2003-54L	26.7	63.6	16.0	2.6
XG98-3-2	23.5	59.6	11.6	3.6
ILL7163	24.2	56.6	10.6	3.1

Seed protein electrophoresis:

In the present study, total seed protein of 10 lentil lines was resolved on SDS-PAGE (Figure 1). A maximum of 22 bands were exhibited in these lines. However, not all of them showed such maximum number of bands. Most bands were common to all the lines. The total number of bands and their respective molecular weight were analyzed. Slight variation was observed in protein profiles among lentil lines, although variation was observed in the density or sharpness of a few bands. These results are in agreement with Malviya *et. al.* (2008) who found no significant variation in protein profiles among accessions of pigeon pea, soya bean, lentil and chickpea, irrespective of the source of germplasm. This indicates that, in addition to total seed storage protein, other proteins, such as 11S, 2S globulins, should be used for studying polymorphism within varieties or within populations of these grain legumes.

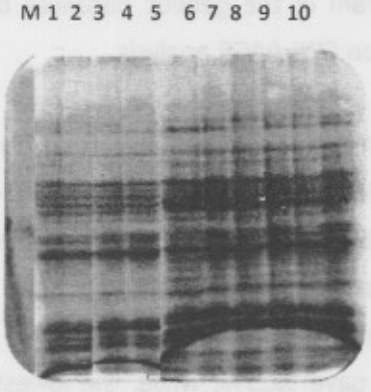


Figure 1. SDS-PAGE of the ten lentil genotypes, Lines from left to right: M= Marker, Giza 9, XG 98-1-1, Giza 51, XG96-5-39, Sinai 1, FLIP 2003-57L, XG 98-9-1, FLIP2003-54L, XG98-3-2, ILL7163.

Relationship among the ten lines were analyzed using SPSS analysis. The similarity ranged from 92.7% to 100% (Table 3). The dendrogram divided the genotypes into two sub cluster where, the first sub cluster included XG98-3-2, ILL7163, FLIP 2003-57L, XG 98-9-1, Sinai 1 and FLIP 2003-54L. while, Giza 51, XG96-5-39, Giza 9 and XG 98-1-1were in the second sub cluster (Figure 2).

Table 3. Similarity matrix among the ten genotypes based on SDS-PAGE analysis.

	Giza 9	XG 98-1-1	Giza 51	XG96-5-39	Sinai 1	FLIP2003-57L	XG 98-9-1	FLIP2003-54L	XG98-3-2
XG 98-1-1	1.000								
Giza 51	1.000	1.000							
XG96-5-39	1.000	1.000	1.000						
Sinai 1	.952	.952	.952	.952					
FLIP2003-57L	.927	.927	.927	.927	.977				
XG 98-9-1	.927	.927	.927	.927	.977	1.000			
FLIP2003-54L	.950	.950	.950	.950	.952	.976	.976		
XG98-3-2	.927	.927	.927	.927	.977	1.000	1.000	.976	
ILL7163	.927	.927	.927	.927	.977	1.000	1.000	.976	1.000

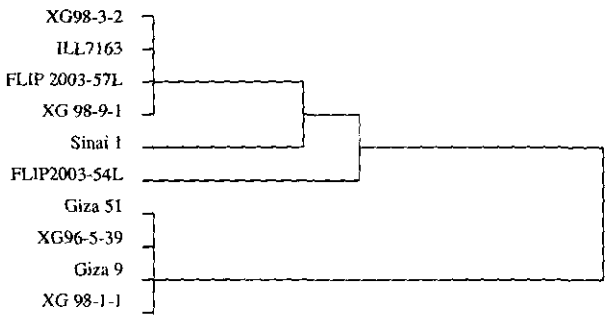
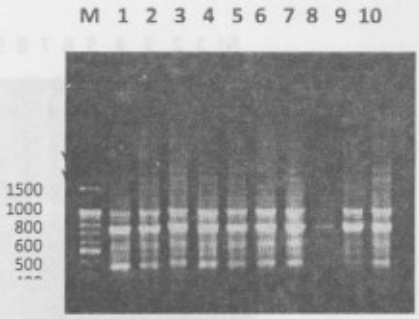


Figure 2. Dendrogram of the genetic distances between the ten lentil genotypes based on SDS-PAGE analysis.

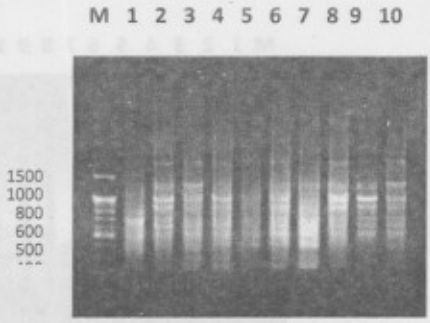
RAPD marker:

Template DNA was extracted from seed samples representing the ten lentil genotypes. Amplification was performed with RAPD-PCR and each of eleven 10-mer primers (Figure 3) which yielded 119 DNA fragments as shown in Table (4). Sixty seven bands of them were polymorphic and fifty two bands were monomorphic (common) for all genotypes. The highest level of polymorphism was observed in primer OP-B7, which gave 92.3%, while, the lowest level of polymorphism (12.5%) was recorded in primer OP-B11.

OP-A01



OP-A08



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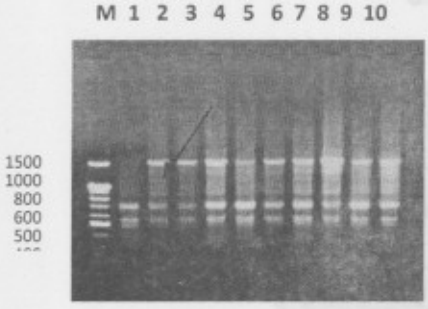
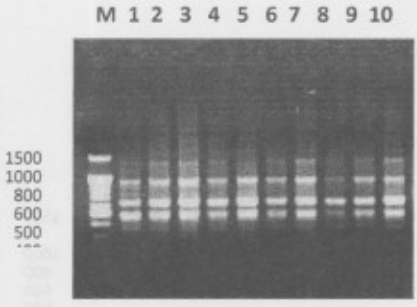
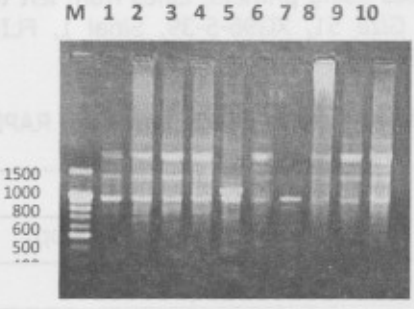


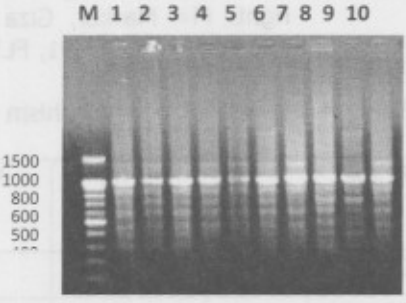
OPA12



OP-B07



OP-B11



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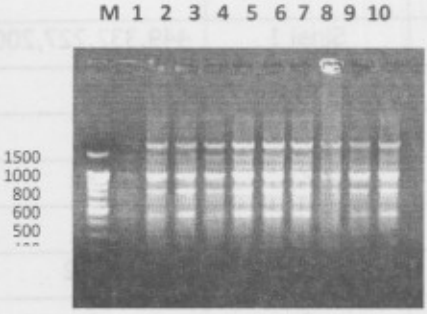
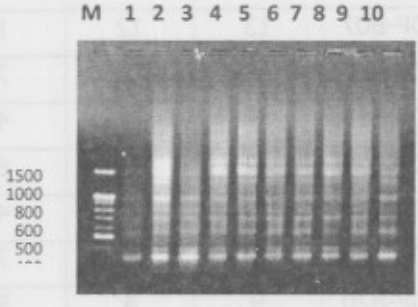


OPB14



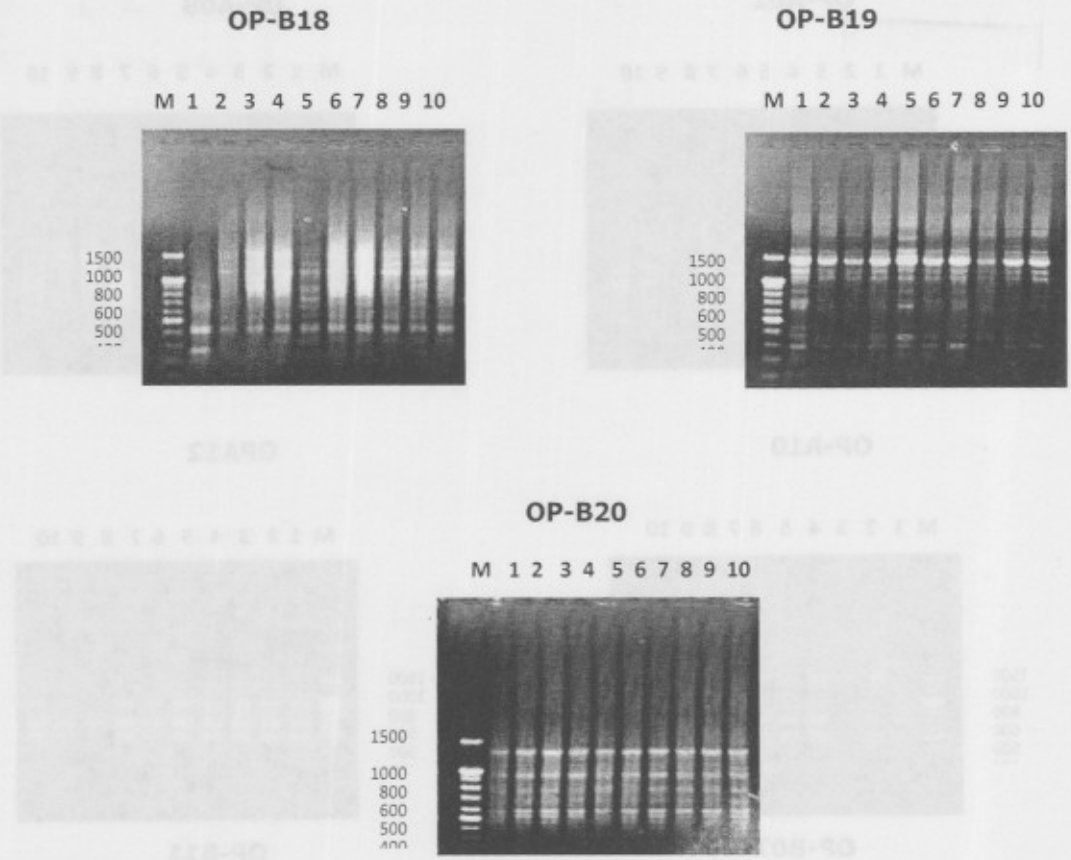


Figure 3. RAPD fingerprinting of the lentil genotypes using primers, Lines from left to right: M= Marker, Giza 9, XG 98-1-1, Giza 51, XG96-5-39, Sinai 1, FLIP 2003-57L, XG 98-9-1, FLIP2003-54L, XG98-3-2, ILL7163.

Table 4. Levels of polymorphism and unique cultivar-specific bands based on RAPD analysis.

Primer	TB	PB	MB	P%	specific bands	
					Cultivar	MS(bp)
OP-A01	11	8	3	72.7	Giza 9	285
OP-A08	13	9	4	69.2	Giza 9	1084, 750
OP-A10	10	3	7	30.0	-	-
OP-A12	8	4	4	50.0	-	-
OP-B07	13	12	1	92.3	Sinai 1	449,332,227,200
OPB11	8	1	7	12.5	-	-
OP-B13	8	4	4	50.0	-	-
OP-B14	12	6	6	50.0	-	-
OP-B18	10	6	4	60.0	-	-
OP-B19	17	10	7	58.8	Sinai 1	2672
OP-B20	9	4	5	44.4	-	-
Total	119	67	52			

MS: Molecular Size, **T.B**: Total bands, **PB**: Polymorphic bands, **MB:** Monomorphic bands and **P%**: Polymorphism%

Only four of the eleven RAPD –PCR primers were found to be useful as specific marker for the genotypes Giza 9 and Sinai 1, where, Giza 9 has three specific markers at 285bp of primer OP-A01 and at 1084, 750bp of primer OP-A08. However, Sinai 1 has five specific markers, four of them observed in primer OP-B07 at 449, 332, 227, 200 bp and one specific marker at 2672bp of primer OP-B19.

RAPDs can produce a large set of markers, which can be used for the evaluation of genetic variations of both between and within species, more rapidly and easily than isoenzymes and microsatellites (Guadognuolo *et. al.,* 2001).

Table (5) shows the genetic similarities based on RAPD analysis among the ten lentil genotypes. The lowest genetic similarity was observed between the genotype Sinai 1 and Giza 9 with 76.8%, while ILL7163 and XG 98-1-1 and also, ILL7163 and Giza 51 had the highest genetic similarity with 95.5%. An unweighted pair-group method with (UPGMA) cluster analysis was performed on the distance matrix using SPSS programme. The dendrogram clearly showed two distinct groups (Figure 4), where Giza 9 was in a separate group while, the second group contains all the other genotypes. This was in agreement with Abdel-Tawab et al. (2006) who found that RAPD-PCR markers seemed to be one of the effective tools for detecting polymorphism and could discriminate between wheat cultivars. RAPDs, however, appear to provide a greater degree of resolution at a sub-species level. The level of variation detected within cultivated lentils suggests that RAPD markers may be an appropriate technology for the construction of genetic linkage maps between closely related *Lens* accessions.

Table 5. Similarity matrix among the ten lentil genotypes based on RAPD analysis.

	Giza 9	XG 98-1-1	Giza 51	XG96-5-39	Sinai 1	FLIP2003-57L	XG 98-9-1	FLIP2003-54L	XG98-3-2
XG 98-1-1	.804								
Giza 51	.802	.939							
XG96-5-39	.820	.944	.923						
Sinai 1	.768	.878	.845	.892					
FLIP2003-57L	.831	.934	.944	.939	.862				
XG 98-9-1	.800	.925	.914	.919	.893	.929			
FLIP2003-54L	.771	.876	.885	.880	.809	.902	.860		
XG98-3-2	.805	.922	.921	.927	.859	.906	.876	.867	
ILL7163	.802	.955	.955	.950	.884	.930	.941	.872	.918

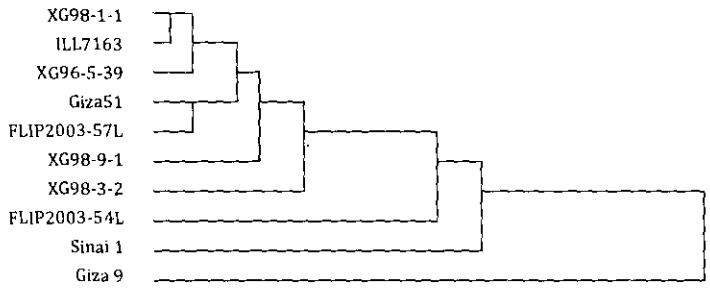


Figure 4. Dendrogram of the genetic distances between the ten Lentil genotypes based on RAPD analysis.

Overall combined class patterns

The similarity matrix among the ten lentil lines based on combined analysis of SDS-PAGE and RAPDs is shown in Table (6), which revealed that the highest similarity was between XG96-5-39 and XG 98-1-1, which were 95.4% followed by 95% between ILL7163 and each of XG 98-1-1, Giza 51 and XG 98-9-1. ,while, the lowest similarity was 80.4% which can observed between Giza 9 and Sinai 1.

The dendogram (Figure 5) separated the ten lentil genotypes into two main sub clusters. Where Giza 9 was in a separate sub cluster while, the second sub cluster contains all the other genotypes. These results were similar with those of RAPD analysis. This was in agreement with Guodagnuolo et al. (2001) who found that the dendrogram obtained using combination of the three systems, isozymes, RAPDs and microsatellites showed a higher similarity in those based on RAPDs and isozymes than the one based on microsatellites. Also, Sun et al. (1999) suggested that the combination of different kinds of markers to detect genetic diversity could be more useful and perhaps less laborious than searching for the most polymorphic type of markers. Rana *et. al.* (2007) found that all cultivars and landraces of lentil except K-75 and L4076 could be discriminated from one another using combined data for the two techniques RAPD and STMS.

Table 6. Similarity matrix among the ten lentil lines based on combined analysis of SDS-PAGE and RAPDs.

	Giza 9	XG98-1-1	Giza 51	XG96-5-39	Sinai 1	FLIP2003-57L	XG98-9-1	FLIP2003-54L	XG98-3-2
XG 98-1-1	.840								
Giza 51	.839	.949							
XG96-5-39	.853	.954	.936						
Sinai 1	.804	.891	.864	.903					
FLIP2003-57L	.849	.933	.941	.937	.882				
XG 98-9-1	.824	.925	.916	.921	.908	.942			
FLIP2003-54L	.806	.889	.897	.893	.836	. 916	.881		
XG98-3-2	.828	.923	.922	.927	.880	923	.898	.887	
ILL7163	.825	.950	.950	.946	.901	.942	.950	.891	.933

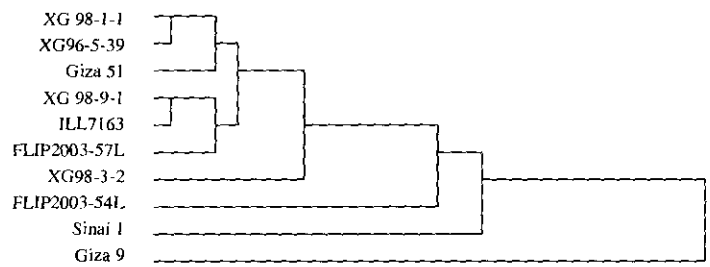


Figure 5. Dendrogram of the genetic distances between the ten lentil genotypes based on combined analysis of SDS-PAGE and RAPDs.

Molecular marker for high iron

The results show one RAPD marker, which was linked to high iron at 1226 bp of primer OP-A10, which was observed in genotypes, XG98-9-1, XG98-1-1 and FLIP2003-54L, which recorded the highest values in iron. These results need further confirmation either by converting RAPD marker into SCAR or by using population specifically designed for this purpose. At this level the resulted RAPD marker may be related to iron content in lentil and could be used as rapid marker to detect and assist in selection for high iron content at early stages of the breeding program as a marker-assisted selection in lentil.

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التوصيف الجزيئى والبيوكيماوى لبعض الطرز الوراثيه من العدس

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أجريت هذه الدراسة بهدف توصيف عشرة طرز وراثية من العدس. تم عمل التحليل الكيماوى لهذه الطرز فكانت نسبة البروتين تتراوح بين ٣٣,٢ % في FLIP2003-57L و ٢٣,٥ % في XG98-3-2 بينما تراوحت نسبة الكربوهيدرات بين ٦٣,٦ % (FLIP2003-54L) و٥٥,٩ %(XG98-9-1). أما مستوى الحديد في هذه الطرز تراوحت من ٤,٧ ملليجم | ١٠٠ جم (Giza9) الى ٢٠,٨ ملليجم | ١٠٠ جم (XG98-9-1) بينما كان مستوى الزنك بين ٢,٦ ملليجم لكل ١٠٠ جم (FLIP2003-54L) ٤,٨ ملليجم | ١٠٠ جم (XG98-9-1).

درست الأختلافات الوراثية بين هذه الطرز الوراثية باستخدام RAPD-DNA و SDS-PAGE. حيث تم أستخدام ١١ بادىء عشوائي نتج عنه ١١٩ حزمة كانت ٦٧ حزمة منهم متباينه (٥٦,٣٠ %). تم قياس درجة القرابة الوراثية باستخدام طريقة Dice والتى أظهرت نسبة من الأختلافات الوراثية بين هذه الطرز. لوحظ أن أعلى نسبة تشابه كان بين ILL7163 وكلا من XG 98-1-1 و Giza51 بنسبة تشابه ٩٥,٥ % بينما لوحظت أعلى أختلافات بين Sinai 1 و Giza 9 بنسبة ٢٣,٢%. أختبارRAPD أنتج عدد من المعلمات التى تعتبر مميزه لبعض الطرز الوراثيه. التفريد الكهربى لبروتينات البذرة الكلية لم تظهر أختلافات فى البروتين بين هذه الطرز. تحليل المجموعات الكلية أظهرت مجموعتين منفصلتين, حيث أن المجموعة الاولى تضم Giza 9 فقط بينما باقى الطرز الوراثية تنضم الى المجموعه الثانية. التركيب Giza 9 كان منفصلا عن باقى الطرز فى التحليل الفردى والكلى. متوسط التشابه الوراثى بين هذه الطرز كان ٨٧,٩ % (تراوحت من ٨٠,٤ الى ٩٥,٤). أعلى تشابه كان بين XG 96-5-39 و XG 98-1-1 وكانت ٩٥,٤% يتبعه ٩٥% بين ILL7163 وكلا من XG 98-1-1 و Giza 51 و XG 98-9-1. بينما أقل تشابه كان ٨٠,٤% بين Giza 9 و Sinai 1. نتائج هذه الدراسة تشير الى أن RAPD-DNA و بروتينات البذرة يبدو مناسبان لأظهار الأختلافات الوراثية بين هذه الطرز فى برامج التربية. كما أظهرت النتائج وجود حزمة DNA بحجم ١٢٢٦ bp عند أختبار الطرز الوراثية تحت الدراسة باستخدام البادىء OP-A10 والتى يمكن أن تستخدم كمعلم في الأنتخاب لصفة المحتوى العالى من الحديد اثناء برامج التربية.