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Characterization of Egyptian durum Wheat Genotypes using Biochemical and Molecular Markers

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Abstract

Landraces are considered an important sources of genetic variations. These variations are extremely important in the programs of plant breeding. Five, eight and twelve oligonucleotide primers of Random amplified polymorphic DNA (RAPD), Simple Sequence Repats (SSRs), and Inter-Simple Sequence Repeats (ISSRs), respectively and protein marker (SDS-PAGE) were used in durum wheat samples analysis (eight cultivars and four landraces). Compared to molecular markers used, proteins fingerprint gave the highest polymorphism (81.8%) with two specific bands, while RAPD analyses revealed the lowest percentage of polymorphism 50% with no specific bands, the numbers of alleles ranged from 3 to 5 per primer, with an average of 4 per primer. On the other hand, ISSRs markers gave the highest number of unique bands (7 bands), 40 of 68 bands were polymorphic with 58.8%polymorphism. The numbers of alleles ranged from 3 to 9 per primer, with average of 5.6 per primer. However, SSRs displayed two specific bands and the highest polymorphism, 31 out of 50 bands were polymorphic with 62% polymorphism and the numbers of alleles ranged from 1 to 10 per primer, with average of 6.2 per primer.Among the different types of molecular markers, SSRs is a more accurate and informative marker. The similarity matrix of collective data differs from the similarity matrix of each studied markers (protein, RAPD, ISSRs and SSRs); and the similarity of each studied marker differs from each other. This indicates that each studied marker has a specific characterization in discrimination of studied genotypes depending on the site of genomic DNA amplified. Finally, it can also be said that biochemical and molecular markers could be used either separately or together for genetic diversity studies in wheat. This study could also be helpful in the future for genomic mapping and breeding programs of wheat genotypes.

Keywords:*TriticumDurum*, Landraces, Protein Marker, SSRs, ISSRs, RAPD.

1. Introduction

Durum wheat is one of the leading cereals in the world and the most important human food crop. Two-thirds of the world populations live on wheat grain, and production has to be increased significantly in the next decades. Data from FAOSTAT indicates that the need is still growing, as indicated by the steadily increasing yield since 1961. Therefore, introducing modern cultivars is necessary for the food production of world populations. The logical treatment of this problem is the conservation of cultivars and landraces of these native resources.

The genetic variation of wheat cultivars and landraces has been affected by various factors throughout their evolutionary history. The need for improved wheat production coupled with stagnation in the cultivated area leads to a more efficient and more productive demand for wheat production. Traditional breeding depends on the genetic selection of genotypes obtained from crosses. Genotype × environment interaction is a common problem including time-consumption and costly procedures of phenotyping.

Molecular markers are unaffected by environmental conditions and are detectable in all stages of the plant growth (Dubcovsky, 2004). Genetic markers are important developments in the field of plant breeding (Kebriyaee et al., 2012). Molecular genetic markers are widely used tools in genotyping and species identification. These molecular markers had been used in wheat for detecting genetic diversity, genotype identification, and genetic mapping (Tanyolac, 2003; Malik et al. 2010). Genetic diversity of wheat genotypes is very critical in reducing genetic vulnerability during plant breeding efforts. So, molecular markers provided excellent tools to evaluate the genetic variability (Sofalianet al., 2008). Molecular markers are classified into various groups on the basis of mode of gene action: co-dominant markers as SSRs or dominant markers RAPD and ISSRs. RAPD analysis has been effieient and extensively used to document genetic variation in Triticum (Cao et al., 1998; Czaplicki et al., 2000; Tahir, 2008), suggesting a narrow genetic base. ISSRs marker have been used in studies of genetic kinship, for resolving intra- and intergenomic relationships, genetic diversity of plant populations, and cultivars (Khurana-Kaul et al., 2012; Verma et al., 2013; Velasco-Ramirez et al., 2014). ISSRs markers are highly polymorphic and repeatable even for intra-specific purposes in durum wheat genotypes and could be used for genotypes identification. SSRs provide highly informative markers because they are co-dominant (Gupta et al., 1996; Agrama and Tuinstra, 2003; Muhammad et al ., 2017). The microsatellite markers have utility in detecting polymorphism and estimating genetic diversity of cultivars (Parvin et al.,

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2014; Ayman and Mohamed, 2019). The aim of this research was to investigate the discrimination capacity chemical marker and molecular markers (dominant markers (RAPDs and ISSRs) and co-dominant markers (SSRs) and their effectiveness in establishing genetic variation and relationships among Egyptian durum wheat genotypes (eight Cultivars and four Landraces).

2. Materials and Methods

2.1. PlantMaterials:

Twelve tetraploiddurum wheat genotypes(Triticum durum) 2n = 4X = 28, eight cultivars and four landraces were provided by the Wheat Research Section and Egyptian National Gene Bank, respectively, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt as are shown in Table (1).

Table (1). The twelve tetraploid wheat genotypes and their origins used in this study.

No.	Genotypes	Genotype
1	Baniswef 3	Cultivar
2	Baniswef 4	Cultivar
3	Baniswef 5	Cultivar
4	Baniswef 6	Cultivar
5	Sohag 1	Cultivar
6	Sohag 3	Cultivar
7	sohag 4	Cultivar
8	sohag 5	Cultivar
9	Sohagalmansheah 33	Landraces
10	Sohagalmansheah 34	Landraces
11	Sohagalmansheah 35	Landraces
12	Sohagalmansheah 41	Landraces

2.2. Methods

2.2.1. Biochemical markers (Protein electrophoresisusing SDS-PAGE technique):

Samples of 0.5 g seeds of each genotypewere were used for protein analyses. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed for protein analysis according to Laemmli, 1970. Sample preparation and extraction of seed storage proteins were performed. The marker of the used protein was BLUltraPrestained Protein Ladder (Gene Direct, Cat No. PM001-0500). In this method, 15% Protein separating gel was used. Protein fractionations were performed using the electrophoresis apparatus manufactured by Cleaver, UK. The images were captured by a digital camera (Sony, made in Japan) and transferred directly to the computer and then the protein bands were analyzed by Total Lab program to find out the molecular weight of each band then compare the presence and absence of the band among studied genotypes, and these data were imported in MVSP (Multi-Variant Statistical Package) (Kovach, 1998) to find the similarity matrix and dendrogram (UPGMA, using Nei&Li's coefficient) which reflect the relationships among the studied genotypes.

2.2.2. Molecularmarkes

2.2.2.1. DNA extraction

The plant genomic DNA was isolated from 12 genotypeswheatby using Gene Jet Plant Genomic DNA purification Mini Kits (Thermo scientificK0791).

2.2.2.2. ISSRs analysis

Twelve ISSRs primers were used to characterize the twelve wheat Genotypes depending upon the literature, Table (2). PCR technique was performed in 25 µl volume containing: master mix (Thermo scientific K1081), 10 µl buffer (10 X), 1µl primer (100 pmol), 1µl DNA template (50 ng) and 13 µlwater (nuclease-free). The amplification was carried out in a thermocycler programmed as follows: 1 cycle of 94°C/2min, 35 cycles of (94°C/1min, annealing temperatures as are shown in Tables (2), /2 min and 72°C/2 min),1 cycles 72°C/7 min. The primer annealing degrees varied according to the melting point of each primer. Agarose gel was used for separating the PCR products of amplified DNA fragments by electrophoresis. The agarosegel was prepared by dissolving 1.2 g agarose in 100 mlbuffer including 40 mMTris-acetate and 2 mMNa EDTA. The gel was stained with ethidiumbromide, photographed under UV light, scanned using Gel-Documentation system (Bio-Rad).

Table (2) represents twelve ISSRs primers names, sequences and Melting temperatures to characterize the twelve durum wheat genotypes:

Primer	Sequence (5'-3')	(Tm)
ISSR-2	(AG)8C	50°C
ISSR-3	(GA)8T	55°C
ISSR-4	(CT)8G	52°C
ISSR-5	(CA)6ACAG	46°C
ISSR-6	BDB(TCC)5	48°C
HB12	(CAC)3GC	52°C
844A	5'(CT)8GC 3'	48°C
844B	5' (CT)7A 3'	48°C
17889A	(CA)6 AC	52°C
17889B	(CA)6 GT	41°C
17898BS5	(CA)GA	43°C
HBS11	(GT)6 CC	44°C

* Y=G/C; B=T/G/C; D=A/T/G (Tm): Melting Temperature

2.2.2.3. SSRs analysis

Eight SSRs primers were used to identify the twelve wheat Genotypes, as shown as in Table (3). PCR technique was performed in 25 µl volume containing: master mix (Thermo scientific K1081), 10 µl buffer (10 X), 1µl primer (100 pmol), 1µl DNA template (50 ng) and 13 µl water (nuclease-free). The amplification was carried out in a thermocycler programmed as follows: 1 cycle of 94°C/2min, 35 cycles of (94°C/1min, annealing temperatures as are shown in Tables (3), /2 min and 72°C/2 min),1 cycles 72°C/7 min. The primer annealing degrees varied according to the melting point of each primer. Agarose gel was used for separating the PCR products of amplified DNA fragments by electrophoresis. The agarosegel was prepared by dissolving 1.2 g agarose in 100 ml buffer including 40 mM Tris-acetate and 2 mM

Na EDTA. The gel was stained with ethidiumbromide, photographed under UV light, scanned using Gel-Documentation system (Bio-Rad).

Table 3. Represents eight SSRs primers names, sequences and Melting temperatures to characterize the twelve durum wheat genotypes

Primer	Sequence	Melting Temperature
Bmag13	Forward 5'-AAGGGGAATCAAAATGGGAG-3' Reverse 5'-TCGAATAGGTCTCCGAAGAAA-3'	54°C
MGB391	Forward 5'-AGCTCCTTTCCTCCCTTCC-3' Reverse 5'-CCAACATCTCCTCCTCCTGA-3'	54°C
GMS1	Forward 5'-CTGACCCTTTGCTTAACATGC-3' Reverse 5'-TCAGCGTGACAAACAATAAAGG-3'	55°C
EBmac624	Forward 5'- AAAAGCATTCAACTTCATAAGA-3' Reverse 5'- CAACGCCATCACGTAATA-3'	54°C
Bmag210	Forward 5'ACCTACAGTTCAATAGCTAGTACC-3' Reverse 5'-GCACAAAACGATTACATCATA-3'	54°C
Bmag149	Forward 5'-CAAGCCAACAGGGTAGTC-3' Reverse 5'-ATTCGGTTTCTAGAGGAAGAA-3'	55°C
HVITR1	Forward 5'-CCACTTGCCAAACACTAGACCC-3' Reverse 5'-TTCATGCAGATCGGGCCAC-3'	55°C
Bmac0576	Forward 5'-CAATTGTAGCCTAGCTGGTCG -3' Reverse 5'-GGGTGTATGCAAGTGGGC-3'	54°C

2.2.2.4. RAPD analysis

Five random, 10-mer primers were used for RAPD analysis depending upon the literature (Table 4) to characterize the twelve wheat Genotypes. PCR technique was performed in 25 µl volume containing: master mix (Thermo scientific K1081), 10 µl buffer (10 X), 1µl primer (100 pmol), 1µl DNA template (50 ng) and 13 µlwater (nuclease-free). The amplification was carried out in a thermocycler programmed as follows: 1 cycle of 94°C/2min, 35 cycles of (94°C/1min, annealing temperatures as shown in Tables (4), /2 min and 72°C/2 min),1 cycles 72°C/7 min. The primer annealing degrees varied according to the melting point of each primer. Agarose gel was used for separating the PCR products of amplified DNA fragments by electrophoresis. The agarosegel was prepared by dissolving 1.2 g agarose in 100 ml buffer including 40 mM Tris-acetate and 2 mM Na EDTA. The gel was stained with ethidiumbromide, photographed under UV light, scanned using Gel-Documentation system (Bio-Rad).

Table (4): Represent five RAPD primers sequence to characterize the twelve durum wheat.

Primer	Sequence (5'-3')	Melting Temperature
OPA1	CAGGCCCTTC	50°C
OPA4	AATCGGGCTG	55°C
OPB3	CATCCCCCTG	52°C
OPB10	CTGCTGGGAC	46°C
OPA11	CAATCGCCGT	48°C

2.2.2.5. Statistical analysis

The data were analyzed using total lab Programs. MVSP (Multi-Variant Statistical Package, Kovach 1998) program was used to find the similarity matrix and dendrogram (UPGMA, using Nei&Li's coefficient), which reflects the relationships among the studied genotypes.

3. Results

3.1. Protein analysis:

SDS-PAGE recorded the differences in the seed storage protein depending on the number of bands among twelve durum wheat Genotypesasare shown in Figure (1) and Table (5). The electrophoresis was estimated based on the molecular weight (Mw) of each band represented with a unit of KiloDaltons (kDa). A total number of the band was 22 bands, four of which were monomorphic (18.2%) and 18 were polymorphicbands with 81.8% polymorphism, including two unique bands at MWs 62kDa and 17 kDa appeared in Sohag almansheah 41 and Sohag almansheah 33 genotypes, respectively. The five genotypes Baniswef 3, Baniswef 4, Baniswef 5, Baniswef 6 and Sohag 1gave the highest number of bands (18 bands) at the same loci in their protein patterns, indicating similar genetic background,while Sohag almansheah 34 exhibited the lowest number of bands (6 bands).

Table 5 . Densitometric analysis represents seed storage protein electrophoretic patterns using SDS-PAGE for characterization of twelve durum wheat genotypes

Band number	Molecular weight	Baniswef 3	Baniswef 4	Baniswef 5	Baniswef 6	Sohag 1	Sohag 3	sohage 4	sohag 5	Sohag almansheah 33	Sohag almansheah 34	Sohag almansheah 35	Sohag almansheah 41
1	110	-	-	-	-	-	-	-	-	+	-	+	+
2	78	+	+	+	+	+	-	-	+	+	-	+	+
3	71	+	+	+	+	+	-	-	+	+	-	+	+
4	62	-	-	-	-	-	-	-	-	-	-	-	+
5	60	+	+	+	+	+	-	+	+	+	-	+	+
6	58	+	+	+	+	+	-	+	+	+	-	+	-
7	56	+	+	+	+	+	+	+	+	+	+	+	+
8	49	+	+	+	+	+	+	+	+	+	+	+	+
9	41	+	+	+	+	+	-	-	+	-	-	+	+
10	35	+	+	+	+	+	+	+	+	+	+	+	+
11	31	+	+	+	+	+	-	-	+	-	-	-	+
12	29	+	+	+	+	+	-	-	+	-	-	-	-
13	26	+	+	+	+	+	-	+	+	-	-	-	-
14	25	+	+	+	+	+	-	-	-	+	+	+	+
15	24	+	+	+	+	+	+	+	+	-	+	-	-
16	19	-	-	-	-	-	+	+	-	-	-	-	-
17	17	-	-	-	-	-	-	-	-	+	-	-	-
18	13	+	+	+	+	+	+	+	+	+	+	+	+
19	9	+	+	+	+	+	-	-	+	+	-	-	-
20	8	+	+	+	+	+	-	-	-	+	-	-	-
21	7	+	+	+	+	+	+	+	+	-	-	-	-
22	5	+	+	+	+	+	+	-	+	+	-	-	-
Total bands		18	18	18	18	18	8	10	16	14	6	11	12

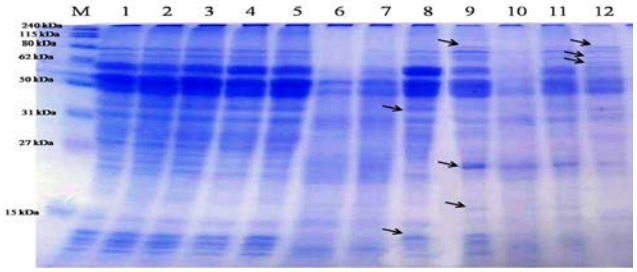


Figure 1. SDS-PAGE of seed storage proteins fingerprint for twelve durum wheat genotypes.

Genetic Similarity: The genetic similarity index and dendrogram tree of the studied twelve durum wheat genotypes were performed using Nei&Li's similarity index on based onproteins electrophoresis as shown in Table (6) and Figure (2). The genetic similarity among 12 the durum wheat genotypes ranged from 42% to 100%, with an average of 71%. Some distinctive genotypes showed identical genetic similarity with others, such as Sohag 1, Baniswef 3, Baniswef 4, Baniswef 5and Baniswef 6 (100%), Sohag5 and (Sohag 1, Baniswef 3, Baniswef 4, Baniswef 5, and Baniswef 6) with similarity 94%, while some varieties displayed low genetic similarity such as Sohag almansheah 41and Sohag 3 revealed 42% similarity, Sohag almansheah 41 and Sohag4 (46%), Sohag almansheah 34 and Sohag 5 (46%). The dendrogram showed that the twelve durum wheat genotypes could be divided into two main clusters. The first cluster was classified into two sub-clusters, the first sub-cluster contained only one genotype Sohag almansheah 34. The second sub-cluster contained two genotypes sohag 3 and sohag 4 with similarity index 82%. The second cluster was divided into two sub-clusters; the first sub-cluster contained two main groups; the first group included Sohag almansheah 41 and Sohag almansheah 35 with similarity 87%, while the second group contained the only one genotypes Sohag almansheah 33. However, the second sub-cluster contained two main groups; the first group included one genotype Sohag5, thes econd group contained the five genotypes Sohag 1, Baniswef 3, Baniswef 4, Baniswef 5, and Baniswef 6 with a similarity of 100%.

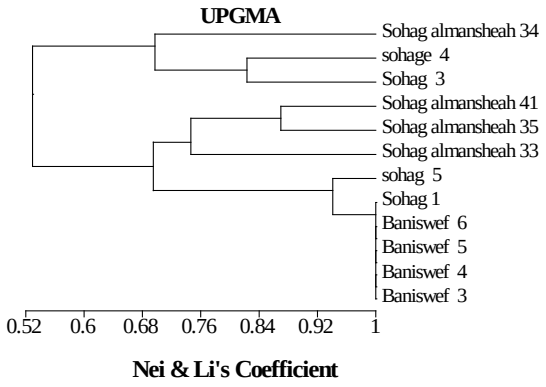


Figure 2. Dendrogram representing the genetic relationship among the twelve durum wheat genotypes using UPGMA cluster analysis of Nei&Lis similarity coefficient generated from seed storage proteins pattern.

Table (6): Genetic similarity percentages of the twelve durum wheat genotypes based on protein banding patterns.

	Baniswef 3	Baniswef 4	Baniswef 5	Baniswef 6	Sohag 1	Sohag 3	sohage 4	sohag 5	Sohag almansheah 33	Sohag almansheah 34	Sohag almansheah 35	Sohag almansheah 41
Baniswef 3	1.00											
Baniswef 4	1.00	1.00										
Baniswef 5	1.00	1.00	1.00									
Baniswef 6	1.00	1.00	1.00	1.00								
Sohag 1	1.00	1.00	1.00	1.00	1.00							
Sohag 3	0.48	0.48	0.48	0.48	0.48	1.00						
sohage 4	0.64	0.64	0.64	0.64	0.64	0.82	1.00					
sohag 5	0.94	0.94	0.94	0.94	0.94	0.52	0.69	1.00				
Sohag almansheah 33	0.75	0.75	0.75	0.75	0.75	0.38	0.58	0.67	1.00			
Sohag almansheah 34	0.50	0.50	0.50	0.50	0.50	0.77	0.63	0.46	0.50	1.00		
Sohag almansheah 35	0.69	0.69	0.69	0.69	0.69	0.44	0.57	0.67	0.80	0.59	1.00	
Sohag almansheah 41	0.67	0.67	0.67	0.67	0.67	0.42	0.46	0.64	0.69	0.56	0.87	1.00

3.2. Identification of durumwheat genotypes by ISSRs analysis:

Twelve ISSRsprimers were used to characterize the twelve durum wheat genotypes. Figure (3) shows the banding patterns produced from each primer for the twelve genotypes. The amplification results indicated distinct differences for the identification of these genotypes as shown in Table (7). A total of 68 amplified DNA fragments ranging in size from173– 1452bp, and 28 fragments out of 68 loci, 41.2% were monomorphic and 58.8 % were polymorphic with average of 3.3 polymorphisms per primer. The number of DNA fragments for each primer varied from 3 (ISSR-5and HBS11) to 9 (17889B) with average of 5.6 fragments per primer. On the other hand, the primer 17889B gave the highest number of amplified fragments (72 fragments) for all studied genotypes, while the primer 844B showed the lowest number of bands (24 fragments). Two specific amplicons with molecular size1452 and 900 bp were detected in the electrophoretic patterns of genotype Sohag almansheah 41 by ISSR2 marker; one specific marker of MW with molecular size1500 bp was detected in genotype Baniswef 3 by HB12 marker. In addition, two specific fragments at molecular sizes 517 and 313 pb revelled in Sohag 1 and Sohag almansheah 41, respectively by 844B marker. Two specific bands with molecular sizes 210 and 500 bp were detected in the genotype Sohag almansheah 41 by 17889B and 1789BS5 markers, respectively. DNA primer 17889 Bout of twelve used in ISSR-PCR analysis succeeded to give the high rate of polymorphism between wheat genotypes.

Table 7. The polymorphic loci amplified by the twelve ISSRs primers

Primer	Loci	range sizes of loci	Polymorphic	Monomorphic	Polymorphism%	Specific bands	Total Bands in all genotypes
ISSR-2	6	800-1452	5	1	83%	2	31
ISSR-3	7	180-650	4	3	57.1%	-	61
ISSR-4	7	183-714	4	3	57%	-	71
ISSR-5	3	173-400	-	3	0%	-	36
ISSR-6	6	219-813	3	3	50%	-	63
HB12	8	383-1519	4	4	50%	1	66
844A	6	200-603	2	4	33.3%	-	59
844B	4	250-512	4	-	100%	2	24
17889A	4	295-718	2	2	50%	-	41
17889B	9	213-715	7	2	77.7%	1	72
17898BS5	5	200-700	4	1	80%	1	35
HBS11	3	291-405	1	2	33.3%	-	28
Total loci	68	173-1452	40	28	58.8 %	7	

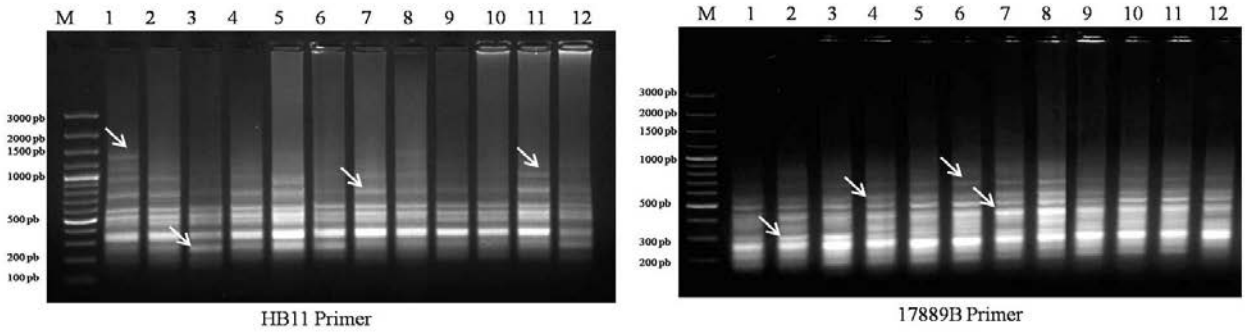


Figure 3. An example of ISSRs banding pattern obtained using HB11and 17889B primers with 12 Egyptian wheat cultivars and landraces. M: ladder. (1) Baniswef 3; (2) Baniswef 4; (3) Baniswef 5; (4) Baniswef 6; (5) Sohag 1; (6) Sohag 3; (7) Sohag 4; (8) Sohag 5; (9) Sohag almansheah 33; (10) Sohag almansheah 34; (11) Sohag almansheah 35;(12) Sohag almansheah 41.

Genetic Similarity: The genetic similarity index and dendrogram tree of studied twelve durum wheat genotypes were performed using Nei&Li's similarity index on the basis ISSR markers in Table (8) and Figure (4). The genetic similarity values ranged from 73 to 98%, with an average of 85.2%. Some distinctive genotypes showed high genetic similarity, while others such as Sohag almansheah 33 and Sohag almansheah 34 gave the highest genetic similarity (98%), Sohagalmansheah 33 and Sohag almansheah 35 (96%). However, some genotypes displayed low genetic similarity such as Sohag almansheah 41 and (Baniswef 3 and Baniswef 4) revealed 73%, Sohag almansheah 41 and Baniswef 5 (75%), Baniswef 6 and Baniswef 3 (73%). The dendrogram resulting from UPGMA cluster analysis showed that the twelve durum wheat genotypes could be divided into two main clusters. The first cluster contained only one genotype Sohagalmansheah41. The second cluster could be divided into two sub-clusters; the first sub-cluster contained two main groups; the first group was classified into two sub-group; the first sub-group included Sohagalmansheah33, Sohagalmansheah34 with similarity (98%), Sohag almansheah 35 and Sohag 5 with similarity (96%), while thes econd sub-group contained only one genotype Sohag 4. The Second-group was divided into two sub-groups; the first sub-group included Sohag1 and Sohag 3 with similarity 93%, the second sub-group had one genotype Baniswef 6. The second sub-cluster consisted of two main groups; the first group included only one genotype Baniswef 5, the second group was composed of the two genotypes Baniswef 3, and Baniswef4 with similarity 85%.

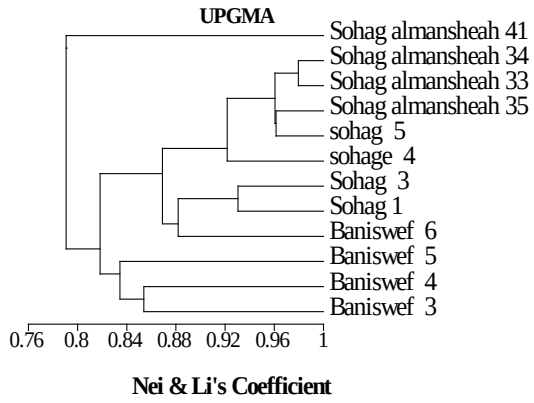


Figure 4. Dendrogram representing the genetic relationship among the twelve durum wheat genotypes using UPGMA cluster analysis of Nei-Lis similarity coefficient generated from ISSRs markers.

Table 8. Genetic similarity percentages of the twelve durum wheat genotypes based on ISSRs banding patterns.

	Baniswef 3	Baniswef 4	Baniswef 5	Baniswef 6	Sohag 1	Sohag 3	sohag 4	sohag 5	Sohag almansheah 33	Sohag almansheah 34	Sohag almansheah 35	Sohag almansheah 41
Baniswef 3	1.00											
Baniswef 4	0.85	1.00										
Baniswef 5	0.84	0.83	1.00									
Baniswef 6	0.73	0.82	0.80	1.00								
Sohag 1	0.82	0.90	0.86	0.89	1.00							
Sohag 3	0.80	0.84	0.84	0.87	0.93	1.00						
sohag 4	0.84	0.86	0.84	0.85	0.90	0.87	1.00					
sohag 5	0.81	0.85	0.81	0.84	0.89	0.84	0.93	1.00				
Sohag almansheah 33	0.76	0.84	0.80	0.85	0.91	0.88	0.91	0.96	1.00			
Sohag almansheah 34	0.78	0.82	0.80	0.83	0.89	0.86	0.91	0.96	0.98	1.00		
Sohag almansheah 35	0.77	0.82	0.79	0.86	0.89	0.86	0.93	0.96	0.96	0.96	1.00	
Sohag almansheah 41	0.73	0.73	0.75	0.78	0.78	0.77	0.82	0.82	0.83	0.83	0.85	1.00

3.3. Identification of durum *Wheat genotypes by SSRs analysis:*

Figure (5) shows the banding patterns produced from each SSRs primer for the twelve durum genotypes. Table (9) illustrates the amplification results by PCR for the studied wheat genotypes with eight SSR primers indicated distinct differences for identification of these genotypes. A total of 50 amplified DNA fragments ranging from 50-1217bp were presented, whereas 19 fragments out of 50 loci, 38 % were monomorphic and 62% were polymorphic with an average of 3.9 polymorphisms per primer. The number of DNA fragments for each primer varied from 1 band (MGB391) to 10 (Bmag149) with average of 6.2 fragments per primer. On the other hand, the primer Bmag149 gave the highest number of amplified fragments (90 bands) for all investigated genotypes, while the primer MGB391 showed the lowest number of amplified fragments (12 amplicons). Two specific bands with molecular sizes (1000 and 359 bp) were recorded in genotype Sohag almansheah 41, using primers BMag149 and Bmag13, respectively. Two DNA primers (Bmag13 and Bmag 210) out of eight gave the highest rate of polymorphism.

Table (9): The polymorphic loci amplified by the eight SSRs primers.

Primer	Loci	Range Sizes of loci	Polymorphic	Monomorphic	Polymorphism%	Specific bands	Total Bands in all genotypes
Bmag13	9	123 -803 pb	7	2	77.7%	1	66
MGB391	1	112pb	0	1	0%	-	12
GMS1	8	79 -1217 pb	6	2	75%	-	66
EBmac624	6	147-509 pb	3	3	50%	-	54
Bmag210	9	63-300 pb	6	3	77.7%	-	75
Bmag149	10	51-1000pb	7	3	70%	1	90
HVITR1	3	59-210 pb	-	3	0%	-	36
Bmac0576	4	50-490 pb	2	2	50%	-	43
Total loci	50	50-1217 pb	31	19	62%	2	442

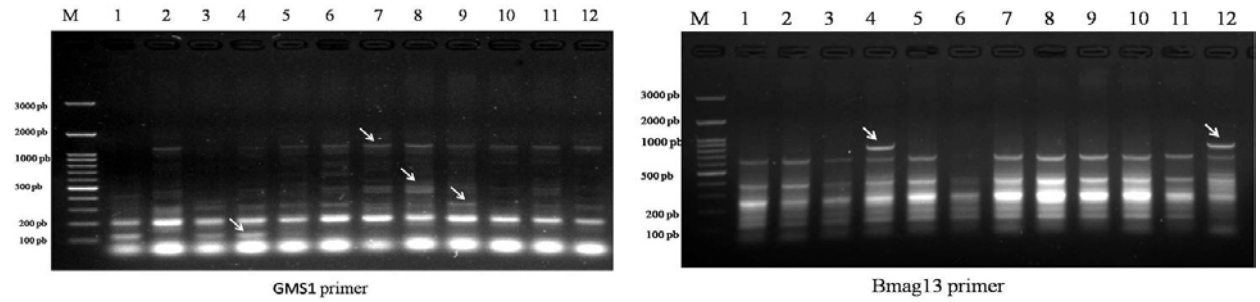


Figure 5. An example of SSRs banding pattern obtained using GMS1 and Bmag 13 primers with 12 Egyptian wheat cultivars and landraces. M: ladder. (1) Baniswef 3; (2) Baniswef 4; (3) Baniswef 5; (4) Baniswef 6; (5) Sohag 1; (6) Sohag 3; (7) Sohag 4; (8) Sohag 5; (9) Sohag almansheah 33; (10) Sohag almansheah 34; (11) Sohag almansheah 35; (12) Sohag almansheah 41.

Genetic Similarity: The genetic similarity index and dendrogram tree of the studied wheat genotypes were performed using Nei-Li's similarity index on the basis SSRs markers in Table (10) and Figure (6). The similarity

values among studied durum wheat genotypes ranged from 64 to 96%, with average 81.5%. Genotypes Sohag3 and Sohag5 gave the highest genetic similarity (96%), Sohag 4 and Sohag 5 (94%). On other hand, Sohag almansheah 41 and Baniswef 3 displayed the lowest genetic similarity (64%), Sohagalmansheah33 and Baniswef 3 (67%). The dendrogram resulting from UPGMA cluster analysis showed that the twelve studied durum wheat genotypes could be divided into two main clusters. The first cluster contained two genotypes Baniswef 3 and Baniswef 5 with similarity 85%, the second cluster was divided into two sub-clusters; the first sub-cluster contained only one genotype, Sohag almansheah 41. The second sub-cluster consisted of two main groups; the first group contained two genotypes, Sohag almansheah 34 and sohag almansheah 35 with similarity 92%. The second group was Classified into two sub-groups: The first sub-group: hasone genotype Sohagalmansheah 33, the second sub-group: involved Sohag 1 and Baniswef 6 with similarity 90%, sohag 3 and sohag 5 (96%), Baniswef 4 and sohag4 (92%).

Table 10. Genetic similarity percentages of the twelve durum wheat genotypes based on SSRs banding patterns.

	Baniswef 3	Baniswef 4	Baniswef 5	Baniswef 6	Sohag 1	Sohag 3	sohag 4	sohag 5	Sohag almansheah 33	Sohag almansheah 34	Sohag almansheah 35	Sohag almansheah 41
Baniswef 3	1.00											
Baniswef 4	0.76	1.00										
Baniswef 5	0.85	0.83	1.00									
Baniswef 6	0.76	0.86	0.80	1.00								
Sohag 1	0.74	0.90	0.81	0.90	1.00							
Sohag 3	0.72	0.90	0.75	0.89	0.88	1.00						
sohag 4	0.74	0.92	0.81	0.89	0.88	0.90	1.00					
sohag 5	0.73	0.91	0.77	0.88	0.90	0.96	0.94	1.00				
Sohag almansheah 33	0.67	0.84	0.74	0.84	0.86	0.82	0.88	0.87	1.00			
Sohag almansheah 34	0.73	0.84	0.77	0.83	0.88	0.85	0.87	0.89	0.79	1.00		
Sohag almansheah 35	0.75	0.81	0.73	0.83	0.84	0.84	0.81	0.83	0.72	0.92	1.00	
Sohag almansheah 41	0.64	0.78	0.70	0.85	0.84	0.79	0.83	0.83	0.76	0.86	0.8	1.00

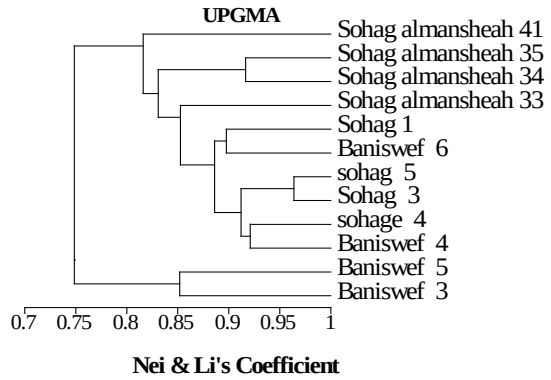


Figure 6. Dendrogram representing the genetic relationship among the twelve durum wheat genotypes using UPGMA cluster analysis of Nei-Lis similarity coefficient generated from SSRs markers

3.4. Identification of durum Wheat Varieties by RAPD analysis:

Characterization of the twelve durum wheat genotypes was performed by five RAPD primers. Figure (7) shows the banding patterns produced from each primer for the twelve genotypes. The amplification results by PCR for the studied wheat genotypes are illustrated in Table (11). A total of 20 amplified DNA fragments ranged from 189– 853bp, whereas ten fragments out of 20 loci, were monomorphic 50 % and 10bansd were polymorphic (50%) with average of 2polymorphic fragments per primer. However, the primer OPB10 gave the highest number of amplified fragments (44 bands) for all studied genotypes, while the primer OPA3 showed the lowest number of amplified fragments. (30amplicons). No specific markers were detected in the electrophoretic patterns of all genotypes. The number of DNA fragments for each primer varied from 3 (OPA3) to 5 (OPB10) with average of 4 fragments per primer.

Table 11. The polymorphic loci amplified by the five RAPD primers.

Primer	Loci	range sizes of Loci	Polymorphic	Monomorphic	Polymorphism%	Specific bands	Total Bands in all genotypes
OPA1	4	200-700pb	3	1	75%	-	35
OPA4	3	300-700 pb	1	2	33.3%	-	30
OPB3	4	312-853 pb	3	1	75%	-	34
OPB10	5	789-189pb	2	3	60%	-	44
OPA11	4	208 -500 pb	1	3	25%	-	41
Total loci	20	189-853pb	10	10	50%	-	184

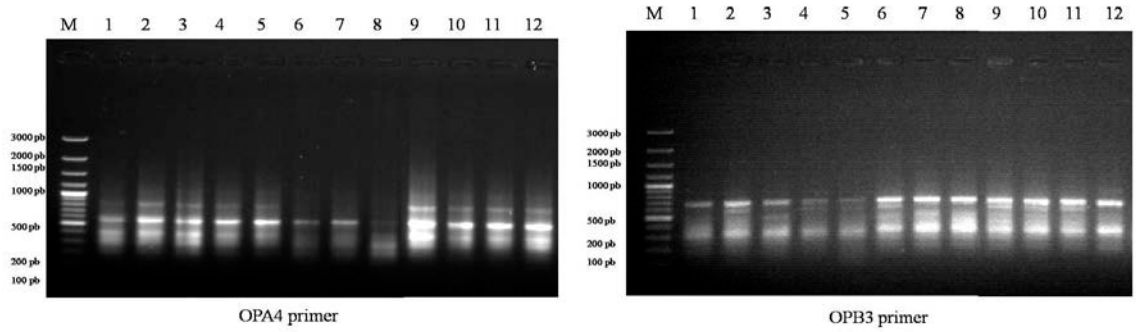


Figure (7): An example of RAPD banding pattern obtained using OPA4 and OPB3 primers with 12 Egyptian wheat cultivars and landraces. M: ladder. (1) Baniswef 3; (2) Baniswef 4; (3) Baniswef 5; (4) Baniswef 6; (5) Sohag 1; (6) Sohag 3; (7) Sohag 4; (8) Sohag 5; (9) Sohag almansheah 33; (10) Sohag almansheah 34; (11) Sohag almansheah 35;(12) Sohag almansheah 41.

Genetic Similarity: The genetic similarity index and dendrogram tree of the studied genotypes were performed using Nei-Li's similarity index depend on RAPDmarkers as shown in Table (12) and Figure (8). The genetic similarity ranged from 54 to 100%, with an average 77%. Sohag almansheah 33 and almansheah 34 showed identical genetic similarity (100%), Sohag1 and Baniswef 4 revealed high genetic similarity (94%), Sohag 4and Baniswef 6 (94%), Baniswef 6 and Baniswef 5 (91%), while some genotypes revealed low genetic similarities such as Sohag3 and Baniswef 3 (54%), Sohag1andsohag 3 (54%), Sohag almansheah 35and Baniswef 3 (59%), Sohag almansheah 41 and Sohag 3 (59%).

The dendrogram resulting from UPGMA cluster revealed that the twelve durum wheat genotypes could be divided into two main clusters. The first cluster contained only one genotype Sohag 3, the second cluster was divided into two sub-clusters; the first sub-cluster contained only one genotype Baniswef 3. The second sub-cluster contained two main groups; the first group was composed of three genotypes, Sohag almansheah 33 and Sohag almansheah 34 with similarity 100% and Sohag almansheah 35. The second group was divided into two sub-groups; the first sub-group included one genotype sohag 5, the second sub-group involved the rest of genotypes.

Table (12): Genetic similarity percentages of the twelve durum wheat varieties based on RAPD banding patterns.

	Baniswef 3	Baniswef 4	Baniswef 5	Baniswef 6	Sohag 1	Sohag 3	sohag 4	sohag 5	Sohag almansheah 33	Sohag almansheah 34	Sohag almansheah 35	Sohag almansheah 41
Baniswef 3	1.00											
Baniswef 4	0.81	1.00										
Baniswef 5	0.8	0.89	1.00									
Baniswef 6	0.77	0.92	0.91	1.00								
Sohag 1	0.80	0.94	0.88	0.97	1.00							
Sohag 3	0.54	0.57	0.61	0.59	0.54	1.00						
sohag 4	0.76	0.86	0.85	0.94	0.91	0.64	1.00					
sohag 5	0.71	0.82	0.81	0.85	0.81	0.58	0.84	1.00				
Sohag almansheah 33	0.74	0.85	0.84	0.81	0.84	0.70	0.73	0.76	1.00			
Sohag almansheah 34	0.74	0.85	0.84	0.81	0.84	0.70	0.73	0.76	1	1.00		
Sohag almansheah 35	0.59	0.85	0.71	0.81	0.84	0.70	0.73	0.76	0.86	0.80	1.00	
Sohag almansheah 41	0.71	0.92	0.86	0.89	0.86	0.59	0.82	0.85	0.81	0.81	0.81	1.00

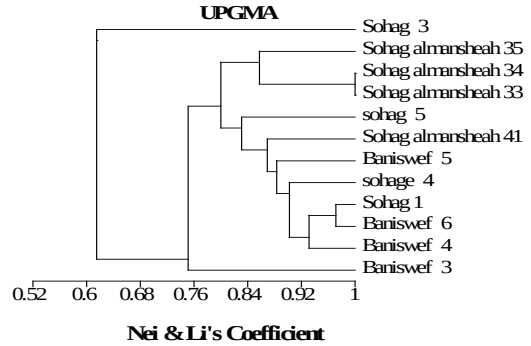


Figure 8. Dendrogram representing the genetic relationship among the twelve durum wheat genotypes using UPGMA cluster analysis of Nei-Lis similarity coefficient generated from RAPD markers.

3.5. Combined results of protein, ISSRs, SSRs and RAPD markers:

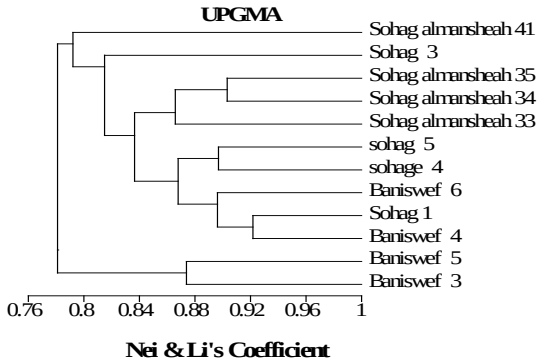
The genetic similarity coefficient and dendrogram tree were gathered between the twelve durum wheat cultivars after protein pattern, ISSRs, SSRs, and RAPD markers as illustrated in Table (13) and Figure (9). The genetic similarity ranged from 70 to 92 %, with average 81%. Sohag1, and Baniswef4 and Baniswef 6 showed the highest genetic similarity (92%), Sohag 4 and Sohag 5 (90%), Sohag almansheah 34 and Sohag almansheah 35 (90%). On the other hand, Sohag almansheah 41 and Baniswef 3 revealed the lowest genetic similarity (70%).

The dendrogram revealed that the twelve durum wheat genotypes could be divided into two main clusters. The first cluster contained only two cultivar Baniswef 3

and Baniswef 5 with similarity 80, the second cluster was divided into two sub-clusters; the first sub-cluster contained only one Laundress Sohag almansheah 41. The second sub-cluster contained two main groups; the first group included only one genotype sohage 3, the second group divided into two sub group; the first sub-group included three landraces Sohag almansheah 35, Sohag almansheah 34 and Sohag almansheah 33, the second sub-group included the rest of genotypes.

Table (13): Genetic similarity percentages of the twelve durum wheat varieties based on combination o proteins, ISSRs, SSRs and RAPD markers.

	Baniswef 3	Baniswef 4	Baniswef 5	Baniswef 6	Sohag 1	Sohag 3	Sohag 4	sohag 5	Sohag almansheah 33	Sohag almansheah 34	Sohag almansheah 35	Sohag almansheah 41
Baniswef 3	1.00											
Baniswef 4	0.84	1.00										
Baniswef 5	0.87	0.87	1.00									
Baniswef 6	0.79	0.87	0.85	1.00								
Sohag 1	0.82	0.92	0.87	0.92	1.00							
Sohag 3	0.71	0.79	0.74	0.80	0.82	1.00						
sohag 4	0.77	0.85	0.81	0.85	0.87	0.85	1.00					
sohag 5	0.80	0.88	0.82	0.87	0.89	0.83	0.90	1.00				
Sohag almansheah 33	0.73	0.83	0.78	0.83	0.86	0.79	0.84	0.87	1.00			
Sohag almansheah 34	0.73	0.80	0.76	0.80	0.84	0.83	0.85	0.86	0.88	1.00		
Sohag almansheah 35	0.73	0.80	0.75	0.82	0.85	0.80	0.83	0.86	0.86	0.90	1.00	
Sohag almansheah 41	0.70	0.77	0.74	0.81	0.80	0.73	0.79	0.80	0.79	0.81	0.83	1.00



Figure(9): Dendrogram generated from the combined results of proteins, ISSRs, SSRs, and RAPD markers among the twelve durum wheat genotypes using UPGMA cluster analysis of Nei-Lis similarity coefficient.

Regarding the collective data of proteins, ISSRs, SSRs and RAPD markers are shown in Table (14), the proteins fingerprint performs the highest percentage of polymorphism 81.8% with two specific bands. On the other hand, RAPD revealed the lowest percentage of polymorphism 50% with no specific bands. ISSRs markers gave (58.8 %) the percentage of polymorphism and gave the highest number of unique bands (7 bands), while SSRs displayed two specific bands and 62% polymorphism.

Table (14): Comparison of the genetic parameter between protein, ISSRs, SSRs and RAPD analysis for the twelve durum wheat genotypes.

Molecular Parameter	Value			
	Protein	ISSR	SSR	RAPD
Total Bands	22	68	50	20
Monomorphic Bands	4	28	19	10
Polymorphic Bands	18	40	31	10
% of Polymorphism	81.8%	58.8%	62%	50%
Unique Bands	2	7	2	-

4. Discussion

We study the genetic diversity of twelve durum genotypes depending on different types of markers, total seed storage protein as a biochemical marker and three types of molecular markers (SSRs, ISSRs, and RAPD markers). The biochemical markers reflect the genetic information of DNA. So, genetic diversity could be efficiently studied using either DNA markers (Mohammadi and Prasanna, 2003) and/or biochemical markers (Nagy *et al.*, 2009). In this study, we observed that the proteins fingerprint provided the highest polymorphism 81.8% with two specific bands, These results were in an agreement with those of (Tahir *et al.*, 1996) who found the high level of polymorphism detected in Pakistani wheat cultivars analyzed with protein markers and (Tahir, 2009) who indicated that protein fingerprint may be useful for selection aims in breeding programs of wheat varieties. Seed storage protein electrophoresis succeeded to identify barley cultivars (El-Rabey*et al.*, 2009b) in maize (Kamal and Yehia, 2010)

On the other hand, RAPD analyses revealed the lowest percentage of polymorphism 50% with no specific bands detected; this percentage of polymorphism was closely related with an earlier study by (Tahir, 2008) who detected the level of polymorphism for bread wheat (40%) and (35%) for durum wheat by RAPD analysis. In RAPD analyses, the number of alleles ranged from 3 to 5 per primer, with average of 4 per primer. The average of polymorphic band (PB) per primer (P) is 2 PB/P; this average was closely related with earlier studies in various plant species such as, 3.58 PB/P in *Cucumis sativus* (Manohar*et al.*, 2013), 2.9 PB/P in bread wheat (Khaled *et al.*, 2015).

However, molecular markers gave different polymorphism average, ISSRs markers represented the highest number of unique bands (7 bands), 40 of 68 bands

were polymorphic with 58.8% polymorphism. The number of alleles ranged from 3 to 9 per primer, with an average of 5.6 per primer. ISSRs markers are highly polymorphic and repeatable even for intra-specific purposes in wheat varieties and could be used for cultivar identification. These results agreed with those of (Abou-Deif*et al.*, 2013) who used Eight ISSRs primers to characterize wheat genotypes including hexaploid, tetraploid and diploid in relation to their genetic background and geographical origin. ISSRs primers produced 112 amplified DNA fragments ranging in size from 127–1857 base pairs, 17 fragments were monomorphic (15.2%) and 95 fragments were polymorphic (84.8%) with average of 11.87 polymorphisms per primer. According to (Fang and Roose, 1997 and Naik *et al.*, 2017), ISSRs markers were found to be more effective in diversity study than RAPD markers. The present investigation clearly supported that view. The variation evidenced by ISSRs markers was due to selective amplification. They amplified conserved regions existing between the microsatellite repeat sequences, but RAPD markers are not selective; rather, they amplified any regions within the entire genome (Zietkiewicz*et al*. 1994). Although major bands from RAPD reactions were highly reproducible, minor bands could pose difficult to repeat due to the random priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers (Tessier*et al.*, 1999).

SSRs displayed two specific bands and the highest polymorphism, 31 out of 50 bands were polymorphic with 62% polymorphism. The number of alleles ranged from 1 to 10 per primer, with average of 6.2 per primer. This result agreed with previous study of (Li *et al*., 2006) who reported that total of 97 alleles were detected at 16 SSR loci. At each locus, the number of alleles ranged from two to fourteen, with an average of 6.1. In wheat, SSRs markers showed a much higher level of polymorphism and informativeness than any other molecular marker (Prasad *et al*., 2000; Wei *et al*., 2003). Therefore, Usefulness Technical in detecting polymorphism and highly variable able to distinguish closely genetically related plant genotypes (Hanaan *et al*., 2013; Ayman and Mohamed, 2019). Highly mutable loci of SSRs may be present at many sites in a genome (Morgante *et al*., 1998). As the flanking sequence of these sites may be unique, primers can be designed to the flanking sequence (Jones *et al*., 1997).

In this study, three of the polymerase chain reaction (PCR)-based systems (RAPD, ISSRs and SSRs). Each system is different in principle, type and amount of polymorphism detected. The level of polymorphism was the highest in SSRs analysis 62% compared with 50% of RAPDs and 58.8% of ISSR (Table 14). These results are in harmony with those of (El-Assal and Gaber, 2012) who found that the highest level of polymorphism appeared by SSRs 83% polymorphism compared with RAPD and ISSR analyses.

Among the different types of molecular markers, SSRs is a more accurate and informative marker because of its co-dominance and stability of results (Gupta *et al.*, 1996; Muhammad *et al.*, 2017). The co-dominant nature of SSR markers also permits the detection of a high number of alleles per locus and contributes to higher levels of expected heterozygosity being reached than would be possible with RAPD markers. However, this result also depends on species under study (Belaj *et al.*, 2003). The highest levels of polymorphism for SSRs system compared to other systems was also reported in previous studies (Russel *et al*., 1997; Rajora and Rahman, 2003; Parvin *et al*., 2014; Ayman and Mohamed, 2019).

The similarity matrix of collective data differs from the similarity matrix of each studied marker (protein, RAPD, ISSRs and SSRs); and the similarity of each studied marker is different. This indicates that each studied marker has a specific characterization in discrimination of studied genotypes depending on the site of genomic DNA amplified. Finally, it can also be said that biochemical and molecular markers could be used either separately or together for genetic diversity studies in wheat.

5. Conclusion

Study the genetic diversity of twelve durum genotypes depending on biochemical (SDS-PAGE) marker and three types of molecular markers (SSRs, ISSRs, and RAPD markers). These markers discriminated most genotypes very effectively, whereas, SSRs markers were more discriminating than RAPD and ISSRs markers. The three molecular markers used in this study have shown an aptitude in the differentiation of the cultivars, the congruence between RAPD, ISSR and SSR data sets suggested that either methods, or a combination of all, are applicable to expend the diversity studies in wheat cultivars. There are different strengths and limitations for marker systems, and knowledge of these may be used to guide the choice of techniques.

Finally it can also be said that molecular and biochemical markers could be used either together or separately for studying the genetic relationships among wheat genotypes.

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