JJBS

Volume 17, Number 4,December 2024
ISSN 1995-6673
Pages 601 – 611
https://doi.org/10.54319/jjbs/170403

Jordan Journal of Biological Sciences

Genetic Differentiation Among Wheat Genotypes Using SDS-PAGE and Molecular Markers

Samira A. Osman*, Samy A.A. Heiba, Hamdy M.H. Abd Elrahman, Rania T. Ali

Genetics and Cytology Department, Biotechnology Research Institute, National Research Centre, Giza, Egypt.

Received: January 21, 2024; Revised: April 9, 2024; Accepted: May 1, 2024

Abstract

Wheat is the most strategic cereal crop worldwide. Studies on the genetic similarities among wheat genotypes are very helpful in the selection of high-quality parents with desirable traits. This study was performed for the discrimination among thirteen wheat genotypes using water-soluble protein, nine Random amplified polymorphic DNA (RAPD) and five Inter simple sequence repeat (ISSR) primers. The maximum genetic similarity percentage was documented between Shandweel1 and Sids12 (96.8%), while the minimum genetic similarity was found between Misr 1 and Sakha 93 (67.7%) based on water-soluble protein profile. On the other hand, the highest percentage of similarty was reported between Sakha 93 and line 18 (95%), while the lowest similarity percentage was found between Gemmeiza 10 and Sids 12 (1.9%), depending on RAPD-PCR. Besides, the highest similarity percentage was recorded between Sakha 93 and Sakha 94 (98.5%), and the least relationship was found between Gemmeiza 9 and sids 12 (66%) based on ISSR-PCR results. RAPD-PCR gave the highest polymorphism (62.35%), followed by ISSR-PCR (54.05%). Finally, SDS-PAGE scored the least polymorphism (35.48%). Despite that, protein analysis provided us with insufficient results, yet it provided us with useful information on the relationships among closely related genotypes and detected the unique band of 13 kDa in the genotype Sids12. Therefore, RAPD and ISSR assays are considered powerful markers for the differentiation among studied wheat genotypes compared with SDS-PAGE.

Keywords: Wheat, Genetic diversity, Biochemical marker, RAPD and ISSR.

1. Introduction:

Triticum aestivum L. (hexaploid wheat, AABBDD, (2n= 6X= 42)) is the most strategic cereal crop worldwide. Wheat grains are rich in proteins (albumins, globulins, prolamins, and glutelins) and carbohydrates, which are used for assessing bread quality (Cooke and Law, 1998; Izadi–Darbandi *et al*., 2010). Developed countries are goals to cultivate wheat crop to afford their population's consumption and trade the excess to developing countries for hard currency. Production of high-quality species is in great need to keep the lead in markets. Studies on the genetic similarities amongst genotypes are very helpful in the selection of high quality parents with desirable traits to breed on a large scale under various agro-climatic conditions and stresses (Qadir *et al*., 2017).

Genetic diversity means the existence of the anchored inherited variation among different varieties within the same species (Salgotra and Chauhan, 2023). Numerous factors influence genetic diversity of plants such as evolutionary factors, mutation, migration, and genetic drift may cause constant changes in allelic frequency. Hence, some morphological, cytological, biochemical, and molecular markers are used for assessing genetic diversity.

Protein banding patterns using the SDS-PAGE technique could be used to analyze the variability of proteins for detecting the phylogenetic relationships and genetic diversity of adapted plant cultivars, improving the productivity of plant breeding programs, and evaluating the genetic diversity among different plant genotypes (Jha and Ohri, 1996; Iqbal *et al*., 2005).

Several molecular markers based on DNA investigation such as RAPD, ISSR, and SSR were utilized to discriminate among different plant genotypes (Gowayed and Abd El-Moneim, 2021; Shaban *et al*., 2022; Abouseada *et al*., 2023). Molecular markers are classified into numerous groups depending on the mode of gene action: co-dominant markers such as SSRs or dominant markers such as RAPD and ISSRs (Souframanien and Gopalakrishna, 2004).

Randomly amplified polymorphic DNA (RAPD) has been widely utilized for determining the genetic difference in *Triticum* because this method is fast, easy to achieve, and inexpensive (Shukre *et al*., 2015). In addition, inter-simple sequence repeats ISSR markers have been performed for resolving intra- and inter-genomic relationships for discrimination of different plant specimens (Khurana-Kaul *et al*., 2012; Velasco-Ramirez *et al*., 2014). ISSR markers are considered reproducible

* Corresponding author. e-mail: s_nrc82@yahoo.com.

** **List of abbreviations**: SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; UPGMA:Unweighted Pair-group Arithmetic ; M: Monomorphic bands; P: Polymorphic bands; kDa: kiloDalton; DNA; deoxyribonucleic acid; PCR: Polymerase chain reaction; RAPD: Random Amplified Polymorphic; DNA ; ISSRs:Inter Simple Sequence Repeats

fingerprinting for assessing genetic variability among wheat genotypes (Najaphy *et al*., 2012; Osman and Ramadan, 2020).

The present study is carried out to detect the genetic relationships among thirteen Egyptian bread wheat genotypes using biochemical marker (SDS-PAGE) and two different molecular markers (RAPD and ISSR).

2. Materials and Methods

2.1. Plant Materials

Thirteen hexaploid wheat genotypes (*Triticum aestivum* L.) 2n = 6X = 42, were kindly obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt as listed in Table (1).

Table 1. The Pedigree and year of release for studied thirteen hexaploid wheat genotypes.

Ser. No.	genotypes	genotype	Pedigree	Source	year
1	Sakha94	Cultivar	Opata/ Rayon//Kauz CMBW90Y31800-TOPM-3Y-010M-010Y-1OM-015Y-0Y-0AP-OS	Egypt	2004
2	Sakha93	Cultivar	Sakha 92TR810328 S 88711-S-2S-1S-0S	Egypt	1999
3	Gemmeiza10	Cultivar	Maya74"S"/On//11603/147-/Bb/4/Chat"S"/5/ctow	Egypt	2004
4	Misr2	Cultivar	SKAUZ/BAV92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-OS	Egypt	2011
5	Sids1	Cultivar	HD 2172/Pavon"S"//1158.57/Maya 74"S"	Egypt	1996
6	Sids12	Cultivar	BUC//7C/ALD/5/MAYA74/ON//1160.1473//BB/GLL/4/CHAT"S"/6/MAYA /VUL//CMMH74A.6304/*SX SD70964-SD-1SD-1SD-0SD	Egypt	2007
7	Giza168	Cultivar	MIL/BUC//Seri CM930468-M-0Y-0M-2Y-0B	Egypt	1999
8	Misr1	Cultivar	OASIS/SKAUZ//4*BCN/3/2*PASTOR CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S	Egypt	2010
9	Shandweel1	Cultivar	Site/MO/4/Nac/Th.Ac//3*Pvn/3/Mirlo/Buc CMSS93B00567S-72Y-010M-010Y-010M-3Y-0M-0THY-0SH	Egypt	2011
10	Line12 (Hatcher)	Line	Yuma'PI372129//TAM 200/3/4*Yuma/4/KS91H184/Vista	Colorado, USA	--
11	Line18 (Kofa)	Line	Selection from composite cross *T. dicoccon* alpha-85 5-1	Colorado, USA	--
12	Line20 (Lovrin 34)	Line	Ranin aja 12/Nadodores 63//Lovrin12	Romania	--
13	Gemmeiza9	Cultivar	Ald"S"/Huac"S"//CMH74A.630/5X CGM4583-GM-1GM-0GM.	Egypt	1999

2.2. Protein banding patterns via SDS-PAGE technique:

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970) as modified by Studier (1973). After that, the electrophoresis gel was stained with Coomassie Brilliant Blue dye, and then destained to visualize the protein bands. The images were transferred to the analyzer program (Total Lab program) to detect the molecular weight of each band among the examined genotypes. Data was imported into the SPSS program to determine the similarity matrix and dendrogram (UPGMA, using Jaccard's coefficient), which reflect the relationships among the studied genotypes.

2.3. DNA extraction

Young leaves of randomly selected samples of each genotype (0.1 gm) were used for genomic DNA extraction using (bio-basic kits). The extracted plant genomic DNA was quantified via a NanoDrop 1000 spectrophotometer (Thermo Scientific). 50ng/µl was then used as a DNA template for PCR reaction.

2.3.1. RAPD-PCR amplification

Nine RAPD primers were utilized for RAPD amplification (Table 2). The amplification was performed in 25µl reaction mixture, which contained 2 µl of genomic DNA (50 ng/ µl), 3µl primer (10 *p*mol), 10X *Taq* DNA polymerase reaction buffer (2.5µl), *Taq* DNA polymerase (1.5 units) and dNTPs (200 mm). Thermocycler (PTC-100 PCR version 9.0-USA) was adjusted for PCR amplification using the following program: 94°C for 5 min (Initial denaturation, one cycle), followed by 35 cycles of 94°C for 30 s,42°C for 90 sec., 72°C for 90 Sec, and final extension at 72°C for 2 min.

Table 2. Code and sequences of nine RAPD primers.

Primer code	Sequence (5`→3`)
OPA-02	CAGGCCCTTC
OPA-04	AATCGGGCTG
OPA-07	GAAACGGGTG
OPB-07	GGTGACGCAG
OPB-10	CTGCTGGGAC
OPO-10	TCAGAGCGCC
OPO-13	GTCAGAGTCC
OPO-14	AGCATGGCTC
OPO-19	CAATCGCCGT

2.3.2. ISSR-PCR amplification

PCR reactions were carried out by using ISSR primers (Table 3) according to Zietkiewciz *et al*. (1994). The reaction mixture was standardized to 20 µl (2.5 mM PCR buffer 1X, $MgCl_2$, 1 mM dNTPs, Primer 10 *p*mol, 1 unit *Taq* polymerase, genomic DNA (50 ng/ µl). The following PCR program was used in a DNA Thermocycler (PTC-100 PCR version 9.0-USA). Initial denaturation at 94°C for 5 min (one cycle), followed by 38 cycles of 94°C for 30 sec, 56 °C for 1 min annealing, 72°C for 2 min, and a final extension at 72 °C for 10 min (one cycle) then hold at 4°C.

Table 3. Code and sequences of five ISSR primers.

Primer code	Sequence (5`→3`)
A12	$(GA)_6CC$
UBC-811	$(GA)_8$ AC
UBC-817	$(CA)_8A$
UBC-814	$(CT)_8A$
UBC-815	$(CT)_8G$

2.3.3. Gel electrophoresis

100 bp DNA ladder (Ferments Life Sciences) and PCR products of RAPD and ISSR were separated on 1% and 1.5% (w/v) agarose gels (staining with ethidium bromide), respectively in 1X TAE buffer (Sambrook *et al.,* 1989). A gel documentation system (Biometra - Bio Documentations) was used for visualizing PCR products by UV-transilluminator and photographed and detecting the polymorphism among the studied bread wheat genotypes.

2.3.4. Data analysis

The amplified bands of RAPD and ISSR were scored as (1) for presence and (0) for absence for each of the thirteen wheat samples according to the gel analyzer program to detect positive and negative markers. The similarity coefficients were generated by the SPSS program version 10 (Nie *et al.,* 1975) to construct a dendrogram by the unweighted pair group method with arithmetical average (UPGMA).

3. Results

3.1. Identification of wheat genotypes by SDS-PAGE:

Obvious differences in protein banding patterns via the SDS-PAGE technique appeared in the protein profiles of thirteen wheat genotypes, as shown in Figure (1) and Table (4). The electrophoresis was estimated based on the molecular weights (MWs) of each band, which were represented with a unit of kilo Daltons (kDa). The total number of bands was 31 bands, of which twenty were monomorphic and eleven were polymorphic (35.48% polymorphism), including one -ve unique band of MW 13 kDa, which disappeared in Sids12 cultivar. The highest number of bands (31 bands) was exhibited in Sids12 cultivar, while Sakha93 exhibited the least number of bands (26 bands).

After the analysis of the obtained image of protein profiles via Jaccard coefficient similarity index, the genetic similarity index and dendrogram tree of the studied thirteen wheat genotypes were achieved, as shown in Table (5) and Figure (2). The similarity values showed substantial differences among the studied wheat genotypes. For instance, the similarity of soluble protein profiles ranged from 67.7% to 98.6%, with an average of 83.85%. The highest similarity was recorded between Shandweel1 and Sids12 (96.8%) while the least genetic similarity was recorded between Misr1 and Sakha93 (67.7%).

Dendrogram represents the genetic relationships among the thirteen wheat genotypes using UPGMA cluster analysis generated from protein marker, which ended to 4 subgroups and five solitary genotypes. The four subgroups are: (line 12& Gemmeiza 10 with a similarity coefficient 93.1%), (Giza 168& Sids 1 with similarity coefficient 96.6%), (line 20& Misr 2 with a similarity coefficient 93.1%) and (Shandaweel1& Sids 12 with a similarity coefficient 96.8%).

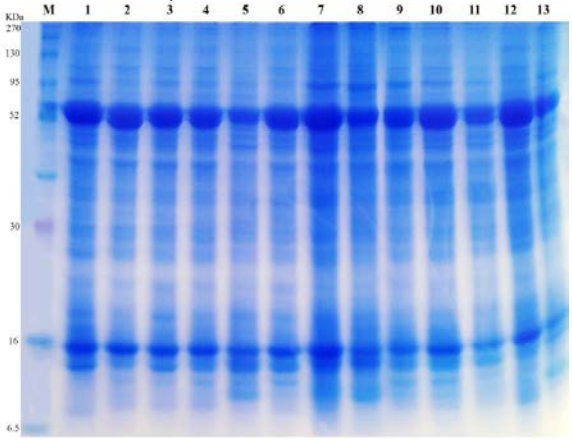

Figure 1. Water soluble proteins banding patterns for thirteen wheat genotypes.

Lane M: Protein marker (270-6.5KDa).

Lane 1: Sakha94. Lane 2: Sakha93. Lane 3: Gemmeiza10. Lane 4: Misr2. Lane 5: Sids1. Lane 6: Sids12. Lane 7: -Giza168. Lane 8: Misr1. Lane 9: Shandweel1. Lane 10: Line12 (Hatcher). Lane 11: Line18 (Kofa). Lane 12: Line20 (Lovrin 34). Lane 13: Gemmeiza9.

Table 4. Banding patterns of water soluble proteins for thirteen wheat genotypes.

Genotype		1	2	3	4	5	6	7	8	9	10	11	12	13
No	MW													
1	253	+	+	+	+	+	+	+	+	+	+	+	+	+
2	209	+	+	+	+	-	+	-	-	+	+	-	+	-
3	135	+	+	+	+	+	+	+	+	+	+	+	+	+
4	125	-	-	-	+	+	+	+	+	+	-	-	+	+
5	115	+	+	+	+	+	+	+	+	+	+	+	+	+
6	108	+	+	+	+	+	+	+	+	+	+	+	+	+
7	95	+	+	+	+	+	+	+	+	+	+	+	+	+
8	82	+	-	-	+	+	+	+	+	+	+	+	+	+
9	56	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
10	46	+	+	+	+	+	+	+	+	+	+	+	+	+
11	43	+	+	+	+	+	+	+	+	+	+	+	+	+
12	41	+	+	+	+	+	+	+	+	+	+	+	+	+
13	40	-	+	+	-	-	+	-	+	-	+	-	-	-
14	38	++	++	++	++	++	++	++	++	++	++	++	++	++
15	36	+	+	+	+	+	+	+	+	+	+	+	+	+
16	35	+	+	+	+	+	+	+	+	+	+	+	+	+
17	34	+	+	+	+	+	+	+	+	+	+	+	+	+
18	31	+	+	+	+	+	+	+	+	+	+	+	+	+
19	29	+	+	+	+	+	+	+	+	+	+	+	+	+
20	28	+	+	+	+	+	+	+	+	+	+	+	+	+
21	25	+	+	+	+	+	+	+	+	+	+	+	+	+
22	21	+	+	+	+	+	+	+	+	+	+	+	+	+
23	20	+	-	+	+	+	+	+	+	+	+	-	+	+
24	18	+	+	+	+	+	+	+	+	+	+	+	+	+
25	17	+	-	+	+	+	+	+	+	+	+	-	-	+
26	15	++	++	++	++	++	++	++	++	++	++	++	++	++
27	14	-	-	-	-	+	+	+	+	+	-	-	-	-
28	13	+	+	+	+	+	+	+	-	+	+	+	+	+
29	12	-	+	+	+	+	+	+	-	+	+	+	+	-
30	10	-	-	-	-	+	+	+	+	+	+	+	+	+
31	9	+	+	+	+	-	+	+	-	+	+	+	+	+
Total bands		26	25	27	28	28	31	29	27	30	29	26	28	27

Lane 1-Sakha94. Lane 2-Sakha93. Lane 3- Gemmeiza 10. Lane 4-Misr2. Lane 5-Sids1. Lane 6-Sids12. Lane 7-Giza168. Lane 8-Misr1. Lane 9-Shandweel1. Lane 10-Line12 (Hatcher). Lane 11-Line18 (Kofa). Lane 12-Line20 (Lovrin 34). Lane 13- Gemmeiza 9.

(-) absent band. (+, ++ and +++) gradual increase in the band intensity.

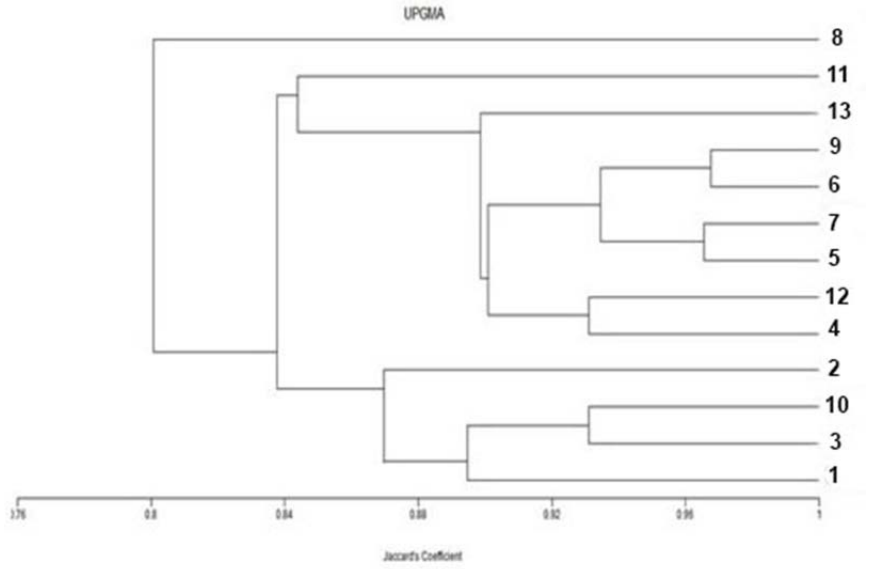

Figure 2. phylogenetic tree using water soluble proteins of thirteen wheat genotypes.

1-Sakha94. 2-Sakha93. 3-Gemmeiza10. 4-Misr2. 5-Sids1. 6-Sids12. 7-Giza168.
8-Misr1. 9-Shandweel1. 10-Line12 (Hatcher). 11-Line18 (Kofa). 12-Line20 (Lovrin 34). 13- Gemmeiza9.

Table 5. proximity matrix of relationship among thirteen wheat genotypes using water soluble proteins.

Case	Sakha 94	Sakha 93	Gemesa 10	Misr2	Sids 1	Sids12	Giza168	Misr1	Shand-weel1	Line12 (Hatcher)	Line18 (Kofa)	Line20 (Lovrin 34)	Gemesa 9
Sakha94	1.000												
Sakha93	0.821	1.000											
Gemmeiza10	0.893	0.926	1.000										
Misr2	0.929	0.828	0.897	1.000									
Sids1	0.800	0.710	0.774	0.867	1.000								
Sids12	0.839	0.806	0.871	0.903	0.903	1.000							
Giza168	0.833	0.742	0.806	0.900	0.966	0.935	1.000						
Misr1	0.767	0.677	0.742	0.774	0.897	0.871	0.867	1.000					
Shandweel1	0.867	0.774	0.839	0.933	0.933	0.968	0.967	0.839	1.000				
Line12 (Hatcher)	0.897	0.862	0.931	0.900	0.839	0.935	0.871	0.806	0.903	1.000			
Line18 (Kofa)	0.821	0.852	0.793	0.828	0.828	0.806	0.862	0.733	0.833	0.862	1.000		
Line20 (Lovrin 34)	0.862	0.828	0.833	0.931	0.867	0.903	0.900	0.774	0.933	0.900	0.893	1.000	
Gemmeiza9	0.893	0.733	0.800	0.897	0.897	0.871	0.931	0.862	0.900	0.867	0.857	0.897	1.000

3.2. Identification of wheat genotypes by RAPD analysis:

Nine of RAPD primers were used to assess relationships among thirteen of Egyptian wheat genotypes. The analysis of results obtained from RAPD revealed differences in number of bands, as shown in Figure (3). The total number of amplified fragments with the nine used primers was 85 bands with 32 monomorphic bands and 53 polymorphic bands to produce 62.35% of polymorphism among the thirteen wheat genotypes (Table 6). The detected band numbers ranged from five bands with OPO-14 primer to 17 bands with OPA-04 primer. The highest percentage of polymorphism was 80% with OPO-14 primer, while the lowest percentage of polymorphism was 5% with OPO-10 primer.

The similarity matrix revealed the highest relationship between Sakha 93 and line 18 with a similarity of 95%. On the other hand, the least relationship was found between Gemmeiza 10 and Sids 12 with a similarity of 1.9% with an average of 48.45% (Table 7). The phylogenetic tree was composed of two clusters; the first one included Gemmeiza10 only, while the second cluster involved all other studied genotypes. The second cluster was divided into two sub-clusters; the first sub-cluster contained Sakha 94 and Sids 1, while the second sub-cluster included the others (ten studied genotypes). The second sub-cluster was divided into two clades; the first clade composed of Giza 168, Shandawel 1, and Line 12 while the second clade involved the remaining seven genotypes. The second clade was divided into two sub-clades; the first sub-clade contained only Sids 12, while the second sub-clade was divided into two groups; the first group included only Misr 1. The second group was divided into two sub-groups; the first sub-group included only Misr 2, while the second sub-group consisted of two sections; the first section included Gemmeiza 9, while the second section included line 18 and Sakha 93 (Figure 4).

Dendrogram represents the genetic relationships among the thirteen wheat genotypes using UPGMA cluster analysis with RAPD primers, which ended to three subgroups (Sakha 93& Line 18) with similarity coefficient 95%, (Shandaweel 1& Line 12) with a similarity coefficient 76.5% and (Sakha 94& Sids 1) with similarity coefficient 66.7%.

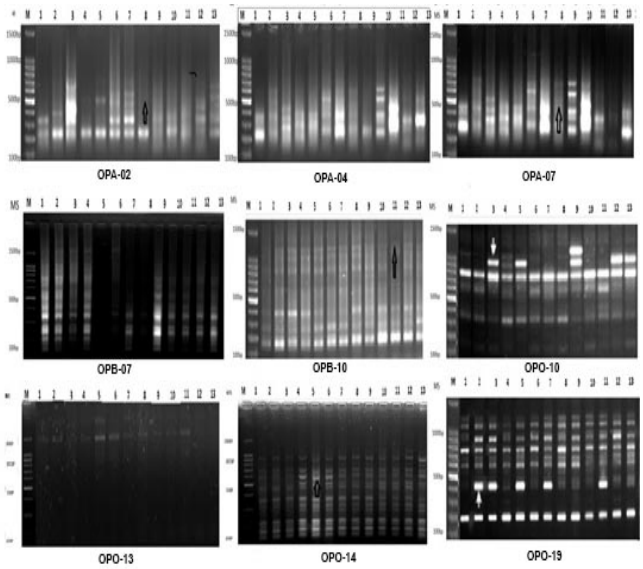

Figure 3.RAPD-PCR banding patterns using RAPD primers for thirteen wheat genotypes.

Lane M: DNA Ladder (100 – 1500 bp).

Lane 1: Sakha94. Lane 2: Sakha93. Lane 3: Gemmeiza10. Lane 4: Misr2. Lane 5: Sids1. Lane 6: Sids12. Lane 7: -Giza168. Lane 8: Misr1. Lane 9: Shandweel1. Lane 10: Line12 (Hatcher). Lane 11: Line18 (Kofa). Lane 12: Line20 (Lovrin 34). Lane 13: Gemmeiza9.

Table 6. Monomorphic and polymorphic bands and %polymorphism for thirteen wheat genotypes using nine RAPD primers.

Code no.	Primer sequences	Monomorphic bands	Polymorphic bands	Total bands	% polymorphism
OPA-02	CAGGCCCTTC	5	7	12	58.3
OPA-04	AATCGGGCTG	6	11	17	64.7
OPA-07	GAAACGGGTG	2	5	7	71.4
OPB-07	GGTGACGCAG	4	5	9	55.6
OPB-10	CTGCTGGGAC	5	6	11	54.5
OPO-10	TCAGAGCGCC	3	3	6	50
OPO-13	GTCAGAGTCC	2	6	8	75
OPO-14	AGCATGGCTC	1	4	5	80
OPO-19	CAATCGCCGT	4	6	10	60
Total		32	53	85	62.35%

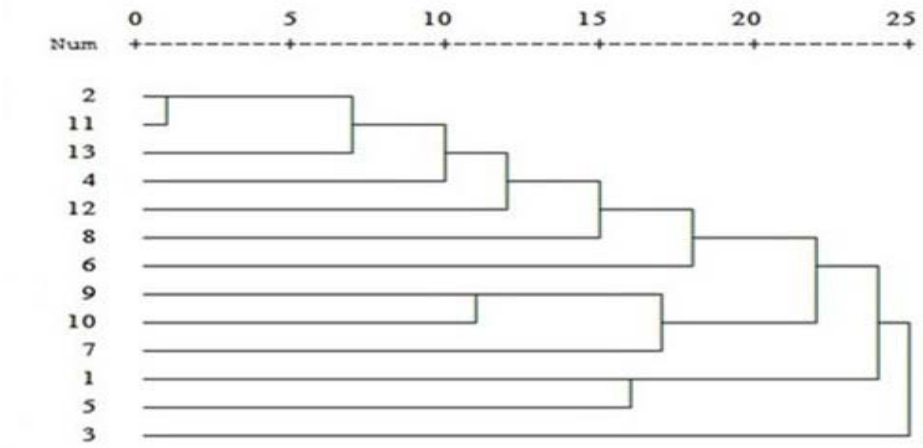

Figure 4. Phylogenetic tree using RAPD-PCR for thirteen wheat genotypes.

1-Sakha94. 2-Sakha93. 3-Gemmeiza10. 4-Misr2. 5-Sids1. 6-Sids12. 7-Giza168. 8-Misr1. 9-Shandweel1. 10-Line12 (Hatcher). 11-Line18 (Kofa). 12-Line20 (Lovrin 34). 13- Gemmeiza9.

Table 7.Proximity matrix of relationship among thirteen wheat genotypes using nine RAPD primers.

Case	Matrix File Input												
	Sakha 94	Sakha 93	Gemmeiza 10	Misr2	Sids1	Sids12	Giza 168	Misr1	Shandweel 1	Line12 (Hatcher)	Line18 (Kofa)	Line20 (Lovrin 34)	Gemmeiza 9
Sakha94	1.000												
Sakha93	0.260	1.000											
Gemmeiza 10	0.260	0.137	1.000										
Misr2	0.468	0.778	0.511	1.000									
Sids1	0.667	0.381	0.392	0.291	1.000								
Sids12	0.033	0.667	0.019	0.260	0.294	1.000							
Giza168	0.532	0.260	0.260	0.319	0.197	.023	1.000						
Misr1	0.468	0.490	0.354	0.552	0.291	0.260	0.617	1.000					
Shandweel 1	0.532	0.552	0.100	0.468	0.197	0.162	0.683	0.468	1.000				
Line12 (Hatcher)	0.468	0.634	0.511	0.552	0.445	0.100	0.468	0.552	0.765	1.000			
Line18 (Kofa)	0.430	0.950	0.319	0.789	0.406	0.532	0.430	0.650	0.430	0.650	1.000		
Line20 (Lovrin 34)	0.713	0.589	0.468	0.650	0.552	0.230	0.571	0.511	0.430	0.511	0.738	1.000	
Gemmeiza 9	0.552	0.713	0.137	0.634	0.230	0.511	0.552	0.778	0.552	0.490	0.863	0.726	1.000

3.3. Identification of wheat genotypes by ISSR analysis:

The discrimination between thirteen wheat genotypes was carried out using five ISSR primers. The PCR products revealed differences in a number of resulting bands, as shown in Figure (5). The total number of bands produced by amplification of all studied primers was 37 bands with 17 monomorphic bands and 20 polymorphic bands to score 54.05% of polymorphism. The highest polymorphism was 62.5% using UBC-817 primer. However, the lowest polymorphism was 37.5% using UBC-815 primer. The number of amplification products generated by primers ranged from 5 (UBC-814) to 9 (A12 primer) bands (Table 8).

UPGMA cluster analysis generated from ISSR marker was translated to generate the dendrogram and similarity index (Figure 6 and Table 9).

The similarity matrix revealed the highest identity between Sakha 93 and Sakha 94 with a similarity of 98.5%. On the other hand, the lowest relationship was found between Gemmeiza 9 and Sids 12 with a similarity of 66% as listed in Table (9). The phylogenetic tree composed of two clusters; the first cluster included only Sakha 94, while the second cluster involved all other studied genotypes. The second cluster was classified to two sub-clusters; the first sub-cluster was divided into two clades, the first clade consisted of Sakha 93 and Line 18, while the second clade involved Gemmeiza 9 and Masr 2. The second sub-cluster was divided into two clades; the first clade composed of two sub-clades; the first sub-clade contained only Misr 1, while the second sub-clade included Sids 1 and Line 20. The second clade was divided into two sub-clades; the first sub-clade included Shandawel 1 only, while the second sub-clade was divided into two groups. The first group included Giza 168 and Sids12, while the second group consisted of Gemmeiza 10 and Line 2 (Figure 6).

Dendrogram represents the genetic relationships among the thirteen wheat genotypes using UPGMA cluster analysis generated from ISSR marker ended to five subgroups and three solitary genotypes. The five subgroups are: (Line 12& Gemmeiza 10 with similarity coefficient 69.4%), (Giza 168 & Sids 12 with similarity coefficient 93.1%), (Line 20& Sids 1 with similarity coefficient75.4), (Line 18& Sakha93 with similarity coefficient 80%) and (Masr 2& Gemmeiza 9 with similarity coefficient 86.5%). While the solitary split genotypes were Sakha 94, Masr 1 and Shandaweel1.

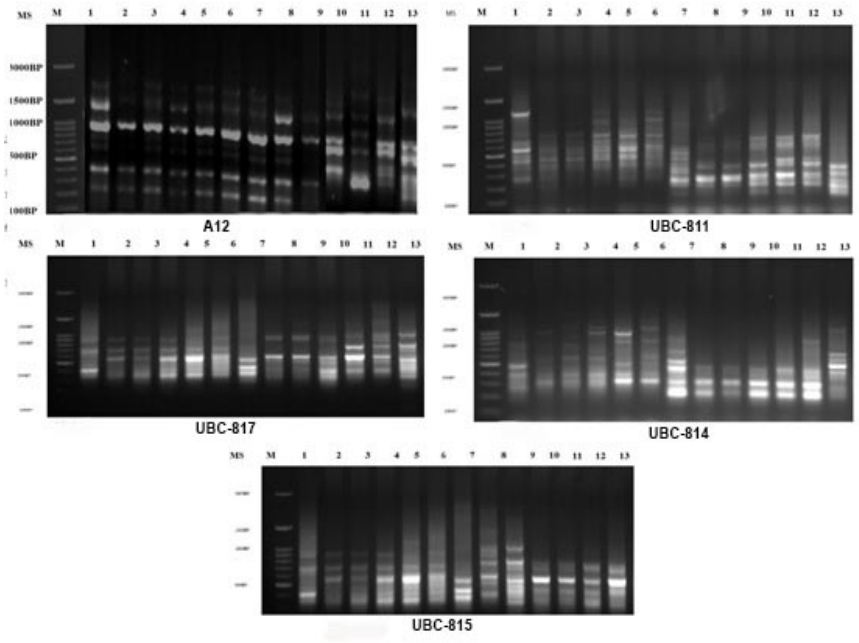

Figure 5. ISSR-PCR banding patterns using RAPD primers for thirteen wheat genotypes.

Lane M: DNA Ladder (100 – 3000 bp).

Lane 1: Sakha94. Lane 2: Sakha93. Lane 3: Gemmeiza10. Lane 4: Misr2. Lane 5: Sids1.

Lane 6: Sids12. Lane 7: -Giza168. Lane 8: Misr1. Lane 9: Shandweel1. Lane 10: Line12 (Hatcher).

Lane 11: Line18 (Kofa). Lane 12: Line20 (Lovrin 34). Lane 13: Gemmeiza9.

Table 8. Monomorphic and polymorphic bands and %polymorphism for thirteen wheat genotypes under study using five ISSR primers.

Primer code	Sequence (5`→3`)	Monomorphic bands	Polymorphic bands	Total bands	% polymorphism
A12	(GA)6CC	4	5	9	55.56
UBC-811	(GA)8 AC	3	4	7	57 14
UBC-817	(CA)8A	3	5	8	62.5
UBC-814	(CT)8A	2	3	5	60
UBC-815	(CT)8G	5	3	8	37.5
Total		17	20	37	54.05%

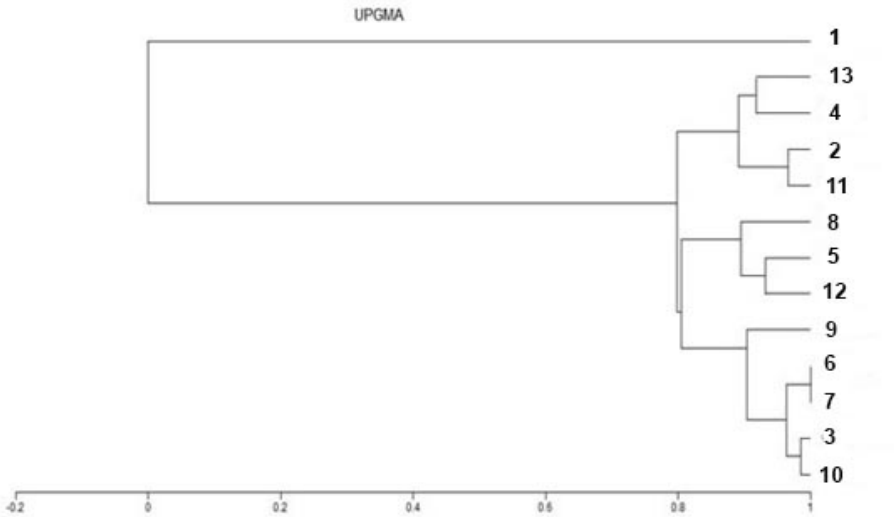

Figure 6. Phylogenetic tree using ISSR-PCR for thirteen wheat genotypes.

1-Sakha94. 2-Sakha93. 3-Gemmeiza10. 4-Misr2. 5-Sids1. 6-Sids12. 7-Giza168.
8-Misr1. 9-Shandweel1. 10-Line12 (Hatcher). 11-Line18 (Kofa). 12-Line20 (Lovrin 34). 13- Gemmeiza9.

Table 9. Proximity matrix of relationship among thirteen wheat genotypes using five ISSR primers

Case	Sakha94	Sakha93	Gemmeiza 10	Misr2	Sids1	Sids12	Giza168	Misr1	Shandweel1	Line12 (Hatcher)	Line 18 (Kofa)	Line20 (Lovrin 34)	Gemmeiza 9
Sakha94	1.000												
Sakha93	0.985	1.000											
Gemmeiza10	0.955	0.971	1.000										
Misr2	0.955	0.971	1.000	1.000									
Sids1	0.875	0.892	0.923	0.923	1.000								
Sids12	0.825	0.844	0.844	0.844	0.918	1.000							
Giza168	0.787	0.806	0.806	0.806	0.847	0.931	1.000						
Misr1	0.721	0.742	0.742	0.742	0.780	0.862	0.929	1.000					
Shandweel1	0.806	0.794	0.794	0.794	0.767	0.780	0.842	0.912	1.000				
Line12 (Hatcher)	0.806	0.794	0.794	0.794	0.733	0.746	0.807	0.877	0.966	1.000			
Line18 (Kofa)	0.813	0.800	0.831	0.831	0.774	0.721	0.780	0.847	0.900	0.933	1.000		
Line20 (Lovrin 34)	0.825	0.844	0.844	0.844	0.754	0.700	0.724	0.793	0.847	0.881	0.918	1.000	
Gemmeiza9	0.780	0.677	0.870	0.865	0.722	0.660	0.730	0.768	0.789	0.765	0.786	0.890	1.000

4. Discussion

Genetic diversity has a critical role in the assessment of the genetic relationships among different plant genotypes by using biochemical markers (Protein electrophoresis) and different molecular markers (Mishra *et al*., 2014).

Protein banding patterns using the SDS-PAGE technique could be considered a reliable tool for the identification and characterization of the similarity among different plant species. This technique was used to analyze the variability of seedling water soluble protein profiles for detecting the phylogenetic relationships and genetic diversity of adapted plant cultivars and improving the productivity of plant breeding programs (Iqbal *et al*., 2005). According to Zahoor *et al. (*2023), genotypes in one cluster should be identical in their protein profile, show less intra-specific genetic variation, and exchange genes smoothly between them, guaranteeing the success of hybrid breeding.

Data obtained from protein profiling as biochemical markers put Line 12 and Gemmeiza 10 in one subgroup, which were highly similar with the exception of three bands missing (82, 20, and 17 kDa) from Gemmeiza 10, two genotypes Giza 168 and Sids 1 were put in one subgroup with high similarity in protein profile with the exception of missing two bands from Giza 168 (207 and 40 kDa), and line 20 and Misr 2 in one subgroup, which have the identical protein fingerprint.

Our obtained data revealed that both protein marker and ISSR marker assessed the genetic similarity coefficient between the two genotypes line 12 and Gemmeiza 10 to reach 93.1 and 79.4%, respectively, and gather them in the same subgroup. The higher induced similarity after protein marker despite the higher genetic diversity on the ISSR molecular marker level may have arisen from paralogue genes (produced to serve the plant evolution) translated into the identical proteins (Koonin, 2005). These paralogue genes expand the genome diversity on the molecular marker side but at the same time summarize the distance among relatives on the protein marker side.

The difference between protein and ISSR was not in the similarity coefficient only but to put different genotypes in different grouping matter. For example, protein electrophoresis gathers Sids 12 and Shandaweel1 in one subgroup with the similarity coefficient 96.8%. At the same time, ISSR marker put Sids 12 and Giza 168 with the similarity coefficient 93.1%.

RAPD-PCR was more precise and informative than SDS-PAGE concerning relationships between *Zea mays* (mays) and *Zea mays* (Mexicana) and between *Sorghum valgare* and *Sorghum bicolor* (Osman *et al*., 2013).

The data recorded herein after the biochemical markers depending on total soluble protein from the thirteen of wheat seedlings agrees with those observed by El-Akkad (1998); El-Akkad and El-Abd El-Kariem (2002), in which they detected genetic variability between and within wheat species and cultivars depending on seed storage protein profiles.

Both of the two molecular RAPD and ISSR markers succeeded to put the two wheat genotypes Sakha 93 and line 18 in same subgroup with a similarity coefficient of 95 and 80% respectively. The difference in resolution of RAPD and ISSR marker systems refers to that the two marker techniques targeted different sequences of the genome.

Comparing the obtained results based on the polymorphism of RAPD and ISSR as molecular markers gave a wide range of the genetic diversity (62.35 and 54.04%, respectively). Therefore, they were considered powerful markers for discrimination among different studied wheat genotypes, compared with biochemical markers (SDS-PAGE) which had the lowest polymorphism (35.48%). This may be explained by that RAPD and ISSR are neutral to environmental influence and indicate variations at the full genome level. Besides, biochemical markers can be affected by extraction methodology, plant

stage, plant tissue, and environmental conditions (Mondini *et al*., 2009).

From the same point of view, our obtained results about the advantage of using molecular markers in assessing the genetic diversity agree with Abdel-Lateif and Hewedy (2018) who recorded that SCoT and ISSR markers were effective in detecting the genetic diversity among different Egyptian wheat and helping breeders to evaluate genetic diversity. Sofalian *et al.* (2008) documented that ISSR markers gave a high value of polymorphism for discrimination of wheat landraces. Also, Pasqualone *et al.* (2000) reported that ISSR markers had productivity, so they used this marker to distinguish all the examined durum wheat cultivars.

Despite that, protein profiling SDS-PAGE of seedling's protein analysis provided us with insufficient results. It provided us with useful information on the relationships among closely related genotypes and detected the unique band at M.W 13 kDa in the genotype Sids12.

In agreement with Abd El-Hady (2010), our results obtained the highest polymorphism using RAPD assays because it targeted the non-coding DNA regions which are very stable, less responsive to external factors, and high tolerance to mutation.

There is a contradiction between our obtained results which revealed the RAPD highest similarity of 95% between (Sakha 93 and line 18) and the least similarity matrix of 1.9% between (Gemmeiza 10 and Sids 12) and the data obtained by Mansour *et al*. (2020) who found the highest similarity matrix between (Sakha 93 and Saheel 1) with a similarity of 86%; while the least similarity matrix was found between (Gemmeiza 9 and Shandaweel 1) with a similarity of 1.9%. This contradiction may be due to the difference in RAPD primers used in their PAPD amplification compared with our used RAPD primers.

5. Conclusions

Thirteen Egyptian wheat genotypes were discriminated using SDS-PAGE, RAPD and ISSR techniques. The discrimination reveals the genetic diversity and, at the same time, points to the relationships between genotypes. RAPD has the highest polymorphism (62.35%), followed by ISSR, which has a polymorphism of 54.05%, and seedling proteins, which have a polymorphism of 35.48%. This means that RAPD and ISSR are more powerful markers than protein marker in the discrimination and identification of different studied wheat genotypes. So, combining the biochemical markers with the molecular markers enriches the study with more informative data. The obtained data can be reliable as a roadmap for the wheat breeders to present more closely related genotypes for estimating new wheat hybrids in great harmony with their surroundings.

Acknowledgement

The authors would like to thank the National Research Centre (Genetics and Cytology Department) Egypt, for providing all the facilities to conduct all experiments in its laboratories. We are thankful to all the researchers whom we cited in this manuscript for their important and beneficial research.

Authors' contributions

Authors S.A.O. and S.A.H. performed RAPD, ISSR, and protein electrophoresis (SDS-PAGE) experiments and analyzed the data. Authors S.A.O. and R.T.A. wrote the manuscript and managed the literature. Author S.A.O. organized the paper. All authors read and approved the final manuscript.

Funding

This research was funded by National Research Centre, Egypt. Project no 11030107.

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