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Estimation of Genetic Diversity among Thirty Bread Wheat Varieties by RAPD Analysis

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Abstract: RAPD markers were used to fingerprint thirty hexaploid wheat varieties (*Triticum aestivum* L.) from different genetic background. A total of 76 DNA fragments were amplified, using five primers, ranging in size from 1884 to 200 base pairs. Out of the 76 amplified products, 19.7% were monomorphic and 80.3% were polymorphic, averaging 12.2 polymorphisms per primer. The number of DNA fragments for each primer varied from 9 (OP-B14) to 20 (OP-C15) with an average of 15.2 fragments per primer. One specific marker out of 11 amplified fragments was detected for primer OP-B11 and two specific markers out of 9 amplified fargments were detected for primer OP-B14. The genetic similarity between varieties ranged from 32 to 97%, with an average of 64.5%. The large genetic diversity may be due to the different areas from which the genotypes were collected. Some distinctive varieties showed high genetic similarity with other varieties, such as Sids1 and Sids 8 (97%), Sids 7 and Icarda 46 (96%), Icarda1 and Gimeza 9 (95%). On the contrary, some varieties displayed low genetic similarity such as Giza164 and Sakha 60 (32%). The similarity values showed clearly substantial differences among the wheat varieties. The dendrogram resulting from the UPGMA cluster analysis showed that the studied varieties could be divided into two main clusters from the same node. The first cluster contained ten varieties four of them are Egyptian, while the second cluster contained 19 varieties including 13 Egyptian. The dendrogram clustered the genotypes into ten groups and showed efficiency in identifying genetic variability. The results indicated that RAPD analysis is useful as molecular genetic marker for estimating the genetic diversity between wheat varieties.

Key words: Hexaploid wheat, Electrophoresis, RAPD, Genetic diversity, Dendrogram

INTRODUCTION

Molecular markers such as RAPD analysis had become popular tools in genetic research for differentiation and determination of phylogenetic relationships among cultivars and detection of genetic differences among species. Bered *et al.*[1] used RAPD marker to evaluate and cluster genetic variability in fifty-four wheat genotypes from different origins and areas. They found that the average genetic similarity value among all genotype pairs was 0.88, showing large genetic relationships in the wheat germplasm evaluated. The dendrogram clustered the genotypes into nine groups and showed efficiency in identifying genetic variability. Cao *et al.*[3] identified twenty-nine common wheat cultivars and assessed their pedigree relationships by RAPD analysis. They used thirty-one primers to generate a total of 214 reproducible amplified DNA fragments. The number of DNA fragments for each primer varied from 3 to 12 with an average of 6.9 bands per primer. The sizes of DNA fragments ranged from 280 to 2800 bp. Out of 214 amplified products, 54.7% were monomorphic and 45.3% were polymorphic, averaging 3.1 polymorphisms per primer.

Mandoulakani *et al.*[9] evaluated the genetic diversity among 28 Iranian wheat cultivars and advanced breeding lines by using RAPD analysis. Fifty decamer primers were used. Eight of these primers amplified template DNA and showed polymorphism among the genotypes. They found that similarity coefficient ranged from 0.40 to 0.91, with an average of 0.64. Dendrogram indicated two main groups. Maric *et al.*[10] used RAPD analysis to estimate genetic diversity among 14 Croatian wheat cultivars and breeding lines. For the RAPD analysis, 36 primers were screened and the 14 most polymorphic ones yielded 341 polymorphic bands. RAPD markers showed a high level of polymorphism among the cultivars examined and the breeding lines. Khan *et al.*[7] examined genetic diversity and relationships among twenty Pakistan wheat cultivars. Forty-two RAPD primers were applied and 184 polymorphic bands were generated for each cultivar. They found that most of

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the cultivars were genetically interrelated, although six of them displayed some genetic distinctness. The RAPD variation observed among these cultivars was low. Only 40.7% of the total scorable bands were polymorphic, and 26.1% of the polymorphic bands were observed most frequently (f = 0.95) among the 20 cultivars.

Muhammad *et al*.[11] estimated genetic diversity/genetic similarity among 9 wheat (*Triticum aestivum*) genotypes using 25 random 10-mer primers, 18 primers detected polymorphism among all the wheat genotypes, while 7 primers produced monomorphic pattern. RAPD data grouped wheat genotypes into 2 main clusters by UPGMA cluster. Bhutta *et al*.[2] used RAPD analysis to estimate the degree of genetic divergence in 7 wheat genotypes from diverse locations of Pakistan. A total of 160 DNA fragments were amplified with 20 random decamer primers with an average of 8 bands per primer. Genetic similarity matrix ranged from 84.0 to 93.0%, which indicated a narrow genetic base among the genotypes. Kobayashi and Yoshida[8] used RAPD analysis to improve the quality of wheat and barley in Tochigi, Japan, and to prevent seed contamination in the foundation and stock seeds. A total of 17 wheat and 19 barley cultivars in the Kanto region were examined, with 5 random primers for wheat and barley cultivars could be identified individually with 6 random primers. A polymorphism was observed among stock seeds collected from various areas.

The objective of this study was to estimate the genetic diversity among thirty hexaploid wheat varieties and fingerprinting them by RAPD analysis.

MATERIAL AND METHODS

Thirty hexaploid wheat varieties (*Triticum aestivum* L.) from different origins and areas that shown in Table (1) were used in this study.

DNA extraction and RAPD-PCR: Ten grains were germinated on wet filter papers for 2 weeks. Genomic DNA was extracted from small amount of young and fresh leaves (0.1 g) from the thirty wheat varieties following the Dellaporta method[4].

PCR reactions were conducted using five arbitrary ten-mer primers with the following sequences: OPB-05: TGCGCCCTTC, OPB-08: GTCCACACGG, OPB-11: GTAGACCCGT, OPB-14: TCCGCTCTGG and OPC-15: GACGGATCAG. PCR technique was performed in 25 µl volume tube containing the following: 10 µl master mix, 10 µl buffer (10 X), 1 µl primer (100 pmol), 1 µl DNA template (50 ng) and 3 µl H2O. The amplification was carried out in a thermocycler (MWG-BIOTECH Primuse) programmed as follow: $(94°C/2')^1$, $(94 °C/1', 37 °C/2', 72 °C/2')^{30}$, $(72 °C/7')^1$.

Agarose gel (1 %) electrophoresis was used for separating the PCR products of amplified DNA fragments. Gels were photographed using a digital camera and scanned with Bio-Rad Video Densitometer Model 620, at a wave length of 577. Software data analyses for Bio-Rad Model 620 USA densitometer and computer programs were used.

Genetic Analysis: Data generated from RAPD analysis were analyzed using the Nei genetic similarity index[12] which excludes common negative data on the basis of the equation, similarity = 2Nab / (Na + Nb), where Nab = number of scored amplification fragments with the same molecular weight shared between genotypes a and b; Na = number of scored amplification fragments in genotype a, Nb = number of scored amplification fragments in genotype b. A dendrogram was constructed on the basis of the similarity matrix data by un-weighted pair group method with arithmetic average (UPGMA) cluster analysis using the software MEGA program.

RESULTS AND DISCUSSION

RAPD analysis using primer OP-B05: The PCR products of this primer revealed 19 amplified DNA fragments with sizes ranged from 200-1884 bp (Fig. 1-A and Table 2). Among such fragments, four common bands at molecular weights of 641, 600, 320 and 250 bp were identified in all studied varieties except the two varieties Giza 164 and Icarda B6. The thirty wheat varieties were characterized according to presence of some distinctive amplified fragments in each variety. The lowest number of amplified fragments was six in the variety Sakha 8, while the highest number was 17 bands in the variety Icarda1. The other varieties displayed different numbers of amplified fragments. RAPD analysis showed that some of the amplified fragments such as 1884 bp were reproducible among few varieties Gimaza 9, Icarda 1, Sids 4 and RCB and fragment with size of 900 bp was presented in two varieties RCB and Maxibak. However, some of the amplified fragments were reproducible in most of the varieties such as fragments with sizes reached 750, 420 and 380 bp.

RAPD analysis using primer OP-B08: The primer OP-B08 produced 17 amplified DNA fragments with sizes ranging from 300-1500 bp (Fig. 1-B and Table 2). RAPD analysis of OP-B08 primer showed 16 polymorphic fragments. The number of total bands varied between varieties where the lowest number was one band presented in the varieties of Giza157 and Sakha 60 at molecular weight 300 bp, while the highest number was 17 bands in Icarda1 variety. There were some fragments found in few varieties,

Table 1: Code numbers, variety names and sources of thirty hexaploid wheat varieties.

No.	variety	Source	No.	Variety	Source
1	Giza 150	Egypt	16	Gimza 5	Egypt
2	Giza 157	Egypt	17	Gimza 7	Egypt
3	Giza 163	Egypt	18	Gimza 9	Egypt
4	Giza 164	Egypt	19	Golan	Syria
5	Giza 168	Egypt	20	Icarda 1	Syria
6	Sakha 8	Egypt	21	Icarda B6	Syria
7	Sakha 60	Egypt	22	Icarda 24	Syria
8	Sakha 69	Egypt	23	Icarda 46	Syria
9	Sakha 93	Egypt	24	Acsad 11	Syria
10	Sakha 202	Egypt	25	Bow	Mexico
11	Sakha 206	Egypt	26	Sonalika	Mexico
12	Sids 1	Egypt	27	KVZ	Mexico
13	Sids 4	Egypt	28	Maxibak	Mexico
14	Sids 7	Egypt	29	MD	Mexico
15	Sids 8	Egypt	30	RCB	Mexico

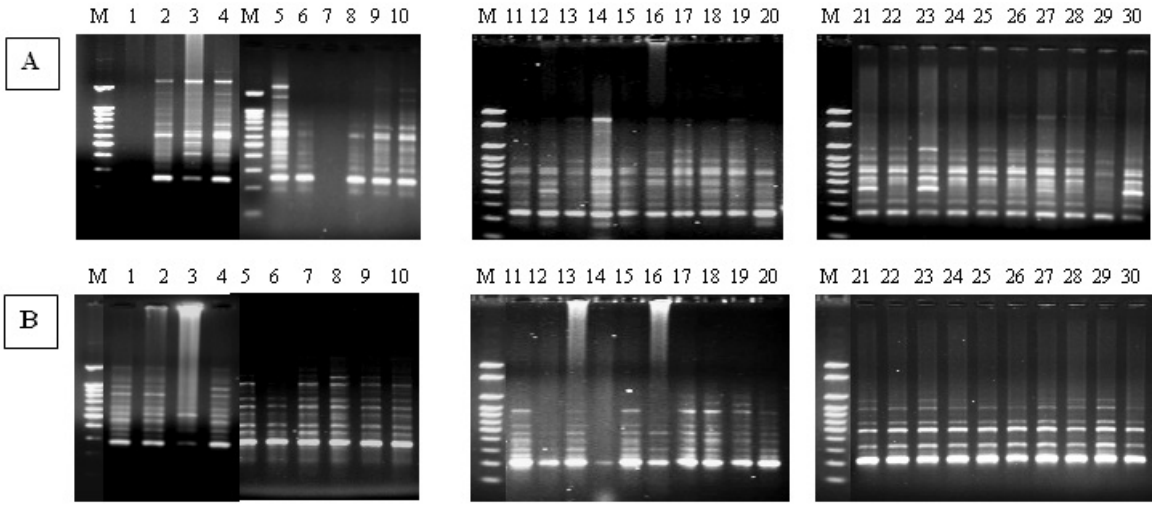

Fig. 1: DNA amplified fragments using (A) OP-B05 and (B) OP-B08 random primers for thirty wheat varieties. M: DNA ladder markers.

such as fragment of size 1500 bp was found in six varieties Giza164, Gimeza 9, Icarda1, Icarda B6, Icarda 24 and MD. Some of the amplified fragments were reproducible in most of the varieties such as fragments with sizes of 891, 670, 587 and 400 bp.

RAPD analysis using primer OP-B11: The results of RAPD marker analysis by using primer OPB-11 for the studied varieties were illustrated in Fig. (2-C) and Table (2). The primer OP-B11 was not used with the variety Giza 150. This primer produced 11 bands distributed in all varieties with molecular weights ranging from 290 to 1295 bp. Two common fragments were found in all varieties at molecular weights of 501 and 601 bp. The varieties of Golan and Icarda 24 revealed the highest number of fragments (10 bands) at the same loci, while Giza 163, Acsad 11 and RCP revealed the lowest number of fragments (two bands)

Table 2: Total bands that were produced from each primer for the wheat varieties

No.	Varieties	Primers					Total bands
		OP-B05	OP-B08	OP-B11	OP-B14	OP-C15	
1	Giza 164	-	16	8	-	15	39
2	Gimeza 9	15	16	8	-	15	54
3	Sakha 69	13	7	8	-	10	38
4	Icarda 1	17	17	8	-	18	60
5	Sids 4	15	12	5	7	20	59
6	Golan	8	7	10	3	20	48
7	Icarda B6	-	13	6	7	10	36
8	Icarda 24	8	14	10	7	12	51
9	KVZ	11	9	6	8	11	45
10	MD	13	11	6	6	19	55
11	Giza 150	8	8	-	-	-	16
12	Giza 157	10	1	3	5	7	26
13	Sakha 8	6	7	3	5	11	32
14	Sakha 60	10	1	3	5	10	29
15	Sakha 93	9	8	3	5	8	33
16	Sakha 202	11	6	3	5	8	33
17	Sids 1	11	11	3	5	6	36
18	Sids 8	9	11	3	5	6	34
19	Giza 163	10	10	2	5	11	38
20	Acsad 11	9	9	2	5	5	30
21	Bow	10	8	6	4	10	38
22	Son	9	8	6	4	8	35
23	Gimeza 5	9	9	6	4	7	35
24	Giza 168	9	8	5	3	10	35
25	Gimeza 7	10	6	3	4	8	31
26	RCB	12	5	2	5	10	34
27	Sids 7	11	8	5	4	7	35
28	Icarda 46	11	8	4	4	7	34
29	Maxibac	9	11	6	5	8	39
30	Sakha 206	8	5	6	4	5	28

at the same loci. Fragment at molecular weight of 380 bp was only presented in the variety MD which is considered a specific marker. Fragment with size 691 bp was presented in three varieties Golan, Icarda 24 and Sakha 8. Some of the amplified fragments were reproducible in most of the varieties; such as fragment with size 320 bp was observed in 25 varieties.

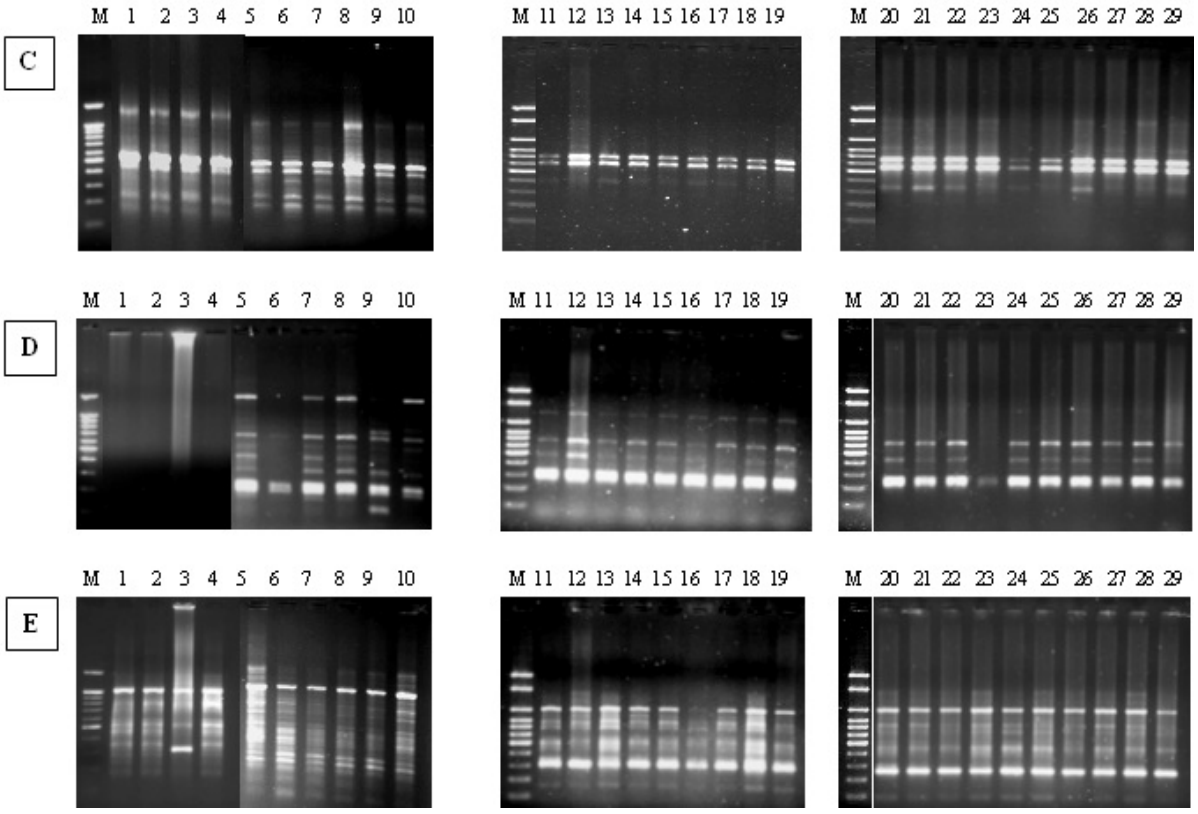

Fig. 2: DNA amplified fragments using (C) OP-B11, (D) OP-B14 and (E) OP-C15 random primers for twenty nine wheat varieties. M: DNA ladder markers.

RAPD analysis using primer OP-B14: Fig. (2-D) and Table (2) manifested the results of RAPD marker analysis by using primer OP-B14 for the studied varieties except Giza150. This primer produced nine amplified DNA fragments distributed in all varieties with molecular weights ranging from 220 to 1251 bp. Three common fragments were presented in 25 varieties at molecular weights of 691, 320 and 220 bp. No fragments were produced in the four varieties of Giza 164, Gimeza 9, Sakha 69 and Icarda 1. Meanwhile, KVZ revealed the highest number of fragments (8 bands), while Golan and Giza 168 gave the lowest number of fragments (three bands) at the same loci. The variety of KVZ showed two specific fragments of molecular weights of 721 and 220 bp, which did not present in the other varieties. However, a fragment of molecular weight of 1251 bp was found in 14 varieties and a fragment with molecular weight of 498 bp was showed in 21 varieties.

RAPD analysis using primer OP-C15: The primer OP-C15 revealed 20 amplified fragments with sizes ranged from 200-1422 bp did not present in all varieties as shown in Fig. (2-E) and Table (2). RAPD analysis of OP-C15 showed 15 polymorphic fragments and five fragments of molecular weights of 1000, 790, 690, 510 and 300 bp were common in all varieties. The number of total bands varied between varieties where the lowest number was five bands present in two varieties Acsad 11 and Sakha 206 at the same loci, and the highest number was 20 fragments in Sids 4 and Golan varieties. There were some fragments characterized few varieties, such as 490 and 390 bp were present in six varieties Giza 164, Gimeza 9, Icarda 1, Sids 4, Golan and MD, while another fragments were present in large number of varieties such as fragments with sizes 1422 and 1238 bp.

The highest number of DNA amplified fragments, using the five primers, was present in variety Icarda 1 (60 amplified fragments), while variety Giza 157 demonstrated the lowest number (26 amplified fragments). The polymorphism revealed by the five primers which used to identify the varieties is shown in Table (3). The primer OP-B08 gave the highest number of polymorphic fragments in all varieties (16 fragments) with 94 % polymorphism while primer OP-B14 gave the lowest number of polymorphic fragments (6 fragments) with 66 % polymorphism. One specific

Table 3: Polymorphisms were revealed by the five primers that used for identification the wheat varieties.

Primer	Total bands	Polymorphic bands	Monomorphic bands	Polymorphism percentage	Specific bands
OP-B05	19	15	4	74 %	-
OP-B08	17	16	1	94 %	-
OP-B11	11	9	2	81 %	1
OP-B14	9	6	3	66 %	2
OP-C15	20	15	5	75 %	-
All primers	76	61	15	80.2 %	3

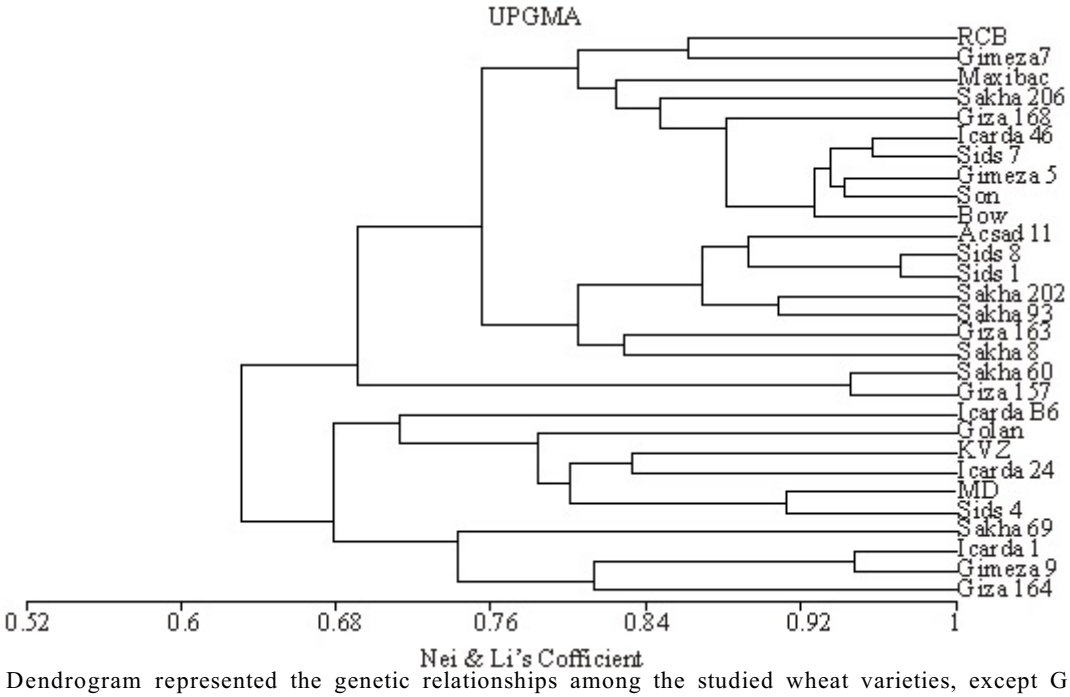

Fig. 3: Dendrogram represented the genetic relationships among the studied wheat varieties, except Giza 150, using UPGMA cluster analysis of Jaccard genetic similarity coefficients generated from five RAPD markers.

marker out of 11 amplified bands was detected for primer OP-B11 and two specific markers out of 9 amplified bands were detected for primer OP-B14, while no RAPD specific markers were detected for the other three primers. Five common fragments were detected for primer OP-C15 and two common fragments for OP-B11. One common fragment was present in the thirty varieties and two common fragments were detected in 28 varieties for primer OP-B08.

The results of amplification by PCR for the wheat varieties with five arbitrary RAPD primers indicated distinct differences for identification of wheat varieties. A total of 76 amplified DNA fragments ranging in size from 1884 to 200 base pairs were presented, whereas 61 fragments were polymorphic and 15 fragments were monomorphic. Therefore, out of 76 amplified products, 19.8 % were monomorphic, and 80.2 % were polymorphic with average of 12.2 polymorphisms per primer. The number of the DNA fragments for each primer varied from 9 (OP-B14) to 20 (OP-C15) with an average of 15.2 bands per primer. These results are in agreement with those of Guadagnuolo *et al*.[6] who reported that RAPDs can produce a large set of markers, which can be used for the evaluation of both between- and within-species genetic variation; and Cao *et al*.[3] who indicated that the RAPD markers are useful in pedigree assessment in common wheat and for the identification of some wheat varieties. Maric *et al*.[10] reported that RAPD markers showed a high level of polymorphism among the cultivars examined and the breeding lines. Also, Bhutta *et al*.[2] used RAPD analysis to estimate the degree of genetic divergence in 7 wheat genotypes from diverse locations of Pakistan. They found that 160 DNA fragments were amplified with 20 random decamer primers with an average of 8 bands per primer.

The dendrogram tree and similarity index of the studied wheat varieties, except Giza 150, were performed using "Nei similarity index" on the basis of RAPD amplified fragments. The similarity values showed clearly substantial differences among the wheat varieties. The genetic similarity ranged from 32 to 97%, with an average of 64.5%. The large genetic diversity that resulted in this study may be due to the different areas from which the genotypes were collected. Some distinctive varieties showed high genetic similarity with each other, such as Sids1 and Sids 8 (97%), Sids 7 and Icarda 46 (96%), Icarda 1 and Gimeza 9 (95%). On the contrary, some varieties displayed low genetic similarity such as Giza 164 and Sakha 60 (32%).

The dendrogram resulting from the UPGMA cluster analysis showed that the studied varieties could be divided into two main clusters from the same node as shown in Fig. (3). First cluster contained ten varieties four of them are Egyptian, while the second cluster contained 19 varieties including 13 Egyptian varieties. The first cluster was divided into two sub-clusters, the first sub-cluster contained four varieties i.e., Icarda 1 and Gimeza 9 (with 95% similarity) grouped with Giza 164 and Sakha 69. The second sub-cluster contained six varieties, two varieties MD and Sids 4 (with 91% similarity) grouped with the two varieties KVZ and Icarda 24 (with 83 % similarity) followed by Golan and Icarda B6.

The second cluster was divided into two sub-clusters; the first contained two Egyptian varieties i.e. Sakha 60 and Giza 157 with 94% similarity, while the second included two groups. The first group contained six Egyptian varieties, Sakha 8 and Giza 163 (with 83% similarity) grouped with Sakha 93 and Sakha 202 (with 91% similarity) followed by Sids 1 and Sids 8 (with 97% similarity) and one Mexican variety (Acsad 11). The second group contained ten varieties, five of them are Egyptian and five are foreign varieties, among such varieties two varieties Sids 7 and Icarda 46 (with 96% similarity) grouped with the two varieties Gimeza 5 and Sonalika (with 94% similarity) followed by Bow, Giza 168, Sakha 206 and Maxibak. The two varieties RCB and Gimeza 7 grouped together with 86 % similarity.

The most Egyptian varieties (13) were belonging to the second cluster indicating that they possessed narrow genetic background. Moreover, six of them were found in one group indicating that they may be produced from one origin genotype. The dendrogram clustered the genotypes into ten groups and showed efficiency in identifying genetic variability.

These results agreed with those of Mandoulakani *et al*.[9] who found that similarity coefficient ranged from 0.40 to 0.91 with an average of 0.64 and the dendrogram indicated two main groups. Also, Muhammad *et al*.[11] found that 18 out of 25 random 10-mer primers detected polymorphism among nine wheat genotypes, and the RAPD data grouped wheat genotypes into 2 main clusters. Similar results were found by Freitas *et al*.[5] who estimated the genetic distances among 14 genotypes of Brazilian wheat and a dendrogram by using RAPD markers. He reported that despite of the low variability found, two groups of genotypes could be identified, which probably reflect their ancestry.

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