Molecular characterization of some Egyptian bread wheat genotypes

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ABSTRACT

The main objective of this investigation was to assess the genetic diversity among five Egyptian bread wheat genotypes (Misr1 and Sids 13 cutlivars and the promising lines No.1, No.2 and No.11) using SDS-PAGE and RAPD markers. The total number of SDS- bands was seven. Six SDS- bands were monomorphic while the other was polymorphic. Line-1 was characterized by the presence of band -3 with a molecular weight of 41.56 kDa. RAPD analysis showed that the number of polymorphic amplicons was 66 out of a total of 93 amplicons, thus revealing a level of 70.97 % polymorphism. The highest genetic similarity revealed by RAPD analysis (93.1%) was between Misr1 and Line 2 genotype. While, the lowest similarity (85.2 %) was between Line 1 and Line 2. The dendrogram separated Line1 from all the other genotypes. The four genotypes constituted a subcluster divided into two groups, one group composed of Misr 1 and Line 2, while the second group comprised Sids 13 and Line 11.

Key words: *Bread wheat, RAPD, Dendrogram, Dice coefficient, Polymorphism , T. aestivum.*

INTRODUCTION

Conventionally, the assessment of the genetic variation in crop plants has been conducted on basis of phenotypic and cytogenetic characters, which frequently lack the resolving power needed to identify individual genotypes (Teshale *et al* ., 2003). Sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is considered as a low cost, reproducible and rapid approach (Laemmli, 1970). In the last decade, molecular markers such as RFLP, RAPD, ISSR, and AFLP have been used to assess genetic variation at the DNA level, allowing an estimation of degree of relatedness between individuals without the influence of environmental variation (Gupta *et al.,* 1999). Randomly amplified polymorphic DNA (RAPD) is a useful method for generating molecular markers (Welsh and McCleland, 1990) that can be used to construct linkage maps, to identify varieties (He *et al.*1992) and to assess genetic diversity (Koller *et al.,* 1993). It is characterized by its low technical input and small quantity of plant DNA needed for the analysis (Hernandez *et al.,* 1999 and Manabe *et al.,* 1999). Also, RAPD based fingerprinting was used successfully in wheat to assess genetic diversity (He *et al.,*1992, Dhaliwal *et al.,*1993; Cao *et al.,*1999; Kudriavtsev *et al.,*2003;

Munshi *et al.*,2003 ; Maric *et al.*, 2004; Abd-El-Haleem *et al.*, 2009 and Hussein *et al.* , 2010). The objectives of this investigation were to: (1) characterize five Egyptian bread cultivars and promising lines at the DNA level using RAPD markers and at protein level using SDS-PAGE and (2) determine the genetic relationships among these genotypes.

Genetic material

In the present study, two Egyptian bread wheat (*Triticum aestivum* L.) cultivars (Misr 1 and Sids 13) and three promising lines (Line 1, Line 2 and Line 11) provided by Wheat Research Dept. of the ARC, Egypt were used.

SDS-PAGE

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the banding patterns of the studied bread wheat genotypes, Seed protein fractionation was performed on vertical slab (16.5 cm x 18.5 cm x 0.2 cm) Hoefer E600, Amersham Pharmacia biotech. according to the method of Laemmli (1970) as modified by Studier (1973). The seed samples were taken from the five wheat genotypes.

RAPD analysis

Extraction and purification of genomic DNA

A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Porebski *et al.* (1997) was adopted for obtaining good quality total genomic DNA. Young green leaves from each genotype were collected from one-week-old seedlings germinated from seeds of each genotype and quickly frozen in liquid nitrogen and then ground using mortar and pestle. Five ml of CTAB extraction buffer (60°C) and 15 µl β-mercaptoethanol (0.3%) were added to a half gram of leaf powder. The tubes were mixed by inversion and incubated at 65°C for one hour. Then, 6 ml of chloroform: isoamyl alcohol (24:1) was added and contents were mixed by inversion to form an emulsion. The tubes were centrifuged at 10,000 rpm for 20 min at room temperature. The top aqueous layer was further centrifuged at 5000 rpm after addition of 6 ml of chloroform: isoamyl (24:1). Half-volume of 5 M NaCl and two volumes of cold absolute ethanol were added to the supernatant and mixed well. The tubes were incubated at – 20°C overnight, then centrifuged at 10,000 rpm for 15 min. The supernatant was discarded; the pellet was washed with 70% cold ethanol, and dried for 10 min. The pellet was dissolved in 300 µl TE buffer (pH 8.0) overnight at 4-6°C.

Estimation of DNA concentration

DNA concentration was determined by diluting the DNA 1:5 in dH_2O. The DNA samples were electrophoresed in 0.7% agarose gel against 10ug of a DNA size marker. This marker covers a range of DNA fragments size between 23130bp and 310bp, and a range of concentrations between 95 ng and 11 ng. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker.

A set of nine random 10-mer arbitrary primers (Table 1) was used in the detection of polymorphism among the five wheat genotypes. These primers were purchased from AGERI. RAPD assay was preformed as described by Williams *et al.* (1990) with some modifications. The amplification reactions were carried out in a volume of 25 µl containing 20ng genomic DNA, 25 pmoles primer, 2mM dNTPs, 2mM $MgCl_2$ and 2 U Taq polymerase (Fermentas) with, 1 x PCR buffer.

Table (1): Sequence of the nine ten-decamer arbitrary primers used in RAPD analysis to detect polymorphism among five wheat genotypes.

No.	Name	Sequence
1	OP-R14	5′ TCCGCTCTGG 3′
2	OP-R17	5′ AGGGAACGAG 3′
3	OP-R20	5′ GGACCCTTAC 3′
4	OP-F11	5′ ACGGATCCTG 3′
5	OP-F14	5′ GGTGATCAGG 3′
6	OP-F15	5′ CCGAATTCCC 3′
7	OP-F16	5′ GGGAATTCGG 3′
8	OP-F18	5′ GGGATATCGG 3′
9	OP-F19	5′ CCAAGCTTCC 3′

Thermocyling profile and detection of the PCR products

PCR amplification was performed in a Perkin-Elmer /GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Polaroid camera. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

RAPD data analysis

The banding patterns generated by RAPD-PCR markers analyses were compared to determine the genetic relatedness of the genotypes. Clear and distinct amplified products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973).

Dice formula: $GSij = 2a/(2a+b+c)$

Where GSij is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called unweighted pair group method using arithmetic average (UPGMA) according to (Sneath and Sokal, 1973).

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SDS- PAGE analysis

SDS banding patterns was used to fingerprint five bread wheat genotypes. Dry wheat seeds were ground into soft flour and water soluble protein fraction was extracted.

The total number of bands was seven bands. Six bands were monomorphic while the other was polymorphic. Line-1 was characterized by the presence of band -3 with molecular weight 41.56 kDa.

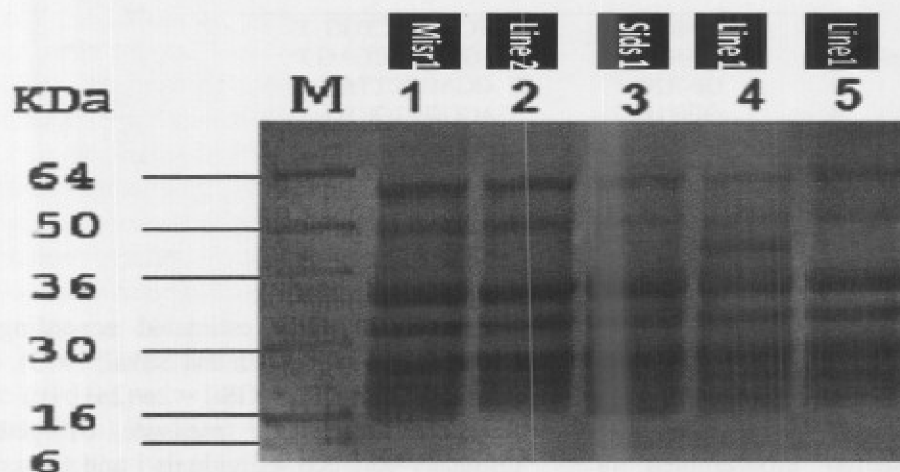

Fig. (1): SDS- PAGE for five wheat genotypes

RAPD analysis

Genetic diversity among wheat genotypes

Different methods are available for analysis of genetic diversity among germplasm accessions. These methods have relied on morphological, agronomic and biochemical data and recently on DNA-based marker data that allow more reliable differentiation of genotypes. In the present investigation, twelve ten-mer arbitrary primers were initially screened for PCR amplification of the genomic DNA for the five wheat genotypes. Only nine primers generated reproducible and easily scorable RAPD profiles. The number of amplified fragments from the genomic DNA of each of the five wheat genotypes generated by the different primers is presented in Table (2).

Table(2):Number of amplified DNA fragments produced by each RAPD primer for the five wheat genotypes.

Primer	Misr1	Line 2	Sids13	Line11	Line1	Total	Mean
OPR14	9	11	8	11	12	51	10.2
OPR17	12	11	12	12	11	58	10.6
OPR20	4	4	6	8	6	28	5.6
OPF11	6	5	4	4	2	21	4.2
OPF14	3	3	5	3	3	17	3.4
OPF15	5	5	3	2	2	17	3.4
OPF16	9	5	9	4	4	31	6.2
OPF18	4	4	3	5	3	19	3.8
OPF19	4	3	3	4	3	17	3.4
Total	56	51	53	53	48	259	82.4
Mean	6.2	5.7	5.9	5.9	5.3	28.8	9.2

Each of the nine primers produced multiple band profiles with the five wheat genotypes. The highest number of amplicons (12 amplicons) was generated by the primer OPR17 in the genomic DNA of the genotype Misr1, Sids13, Line 11 and the primer OPR14 in the DNA of Line1. While, the lowest number of amplicons, i.e., two amplicons were exhibited by primer OPF15 in line 11 and line 1 and primer OPF11 in line 1. which were monomorphic across the five wheat genotypes. As shown in Table (3) the total number of DNA fragments amplified by the nine primers was 93 with an average of 10.33 amplicons per primer. The number of polymorphic amplicons ranged from 1 to 16. Primer OPR17 amplified the highest number of polymorphic amplicons, while, the primer OPF19 revealed the lowest number. Therefore, the different primers expressed different levels of polymorphism, ranging from 25% with primer OPF19 to 84.2% with primer OPR17. The total number of polymorphic bands revealed by the nine primers was 66 and the average number of polymorphic fragments/ primer was 7.3. Thus, the average level of polymorphism was 70. 97 %. The size of amplified fragments varied with the different primers, ranging from 153 to 2406 bp (Figs. 2 and 3).

In this respect, Joshi and Nguyen (1993) investigated the genetic diversity among 15 wheat varieties (*T. aestivum*) using RAPD analysis. Out of 109 amplified DNA fragments, 41 were polymorphic, representing a level of polymorphism of 65%. Perenzin *et al.* (1997) utilized 87 RAPD primers to assay the genetic diversity among wheat genotypes. They reported that 304 polymorphic bands were generated with an average of 3.49 polymorphic amplicon/primer. Sun *et al.* (1998) used 32 arbitrary primers for RAPD analysis of 46 wheat genotypes, among which 26 primers (81.3%) revealed polymorphism. A total of 279 amplicons were generated and 182 (65.2%) were polymorphic. The number of polymorphic amplicons ranged from 2 to 20 with an average of 7 polymorphic amplicons per primer. Zheng *et al.* (2001) used 55 arbitrary primers in the RAPD analysis of 40 wheat cultivars. Out of 183 amplified fragments, 93 amplicons 50.8% were polymorphic, this represented an average of 1.7 polymorphic amplicons per primer. Moreover, Cao *et al.* (2002) screened 235 random primers against four wheat cultivars to detect RAPD polymorphism. Only, 31 (13.20%) primers produced polymorphism these 31 primers generated a total of 214 reproducible amplified fragments when used with 29 common wheat cultivars. The number of amplified fragments produced by each primer varied from 3 to 12 with an average of 6.9 and an average of 3.10 polymorphic band per primer. Al-Naggar *et al.*(2004) used 17 arbitrary primers for RAPD analysis of six bread wheat genotypes. Twelve primers (70.60%) generated polymorphic profiles. The total number of amplicons was 98, of which 34 (34.69%) showed polymorphism. Also, Wjhani (2004) studied the genetic variability among 14 wheat accessions using 39 RAPD primers. The total number of amplicons was 117, including 108 polymorphic amplicons. This represented a level of polymorphism of 92.3% and an average number of 9 polymorphic bands per primer.

species.

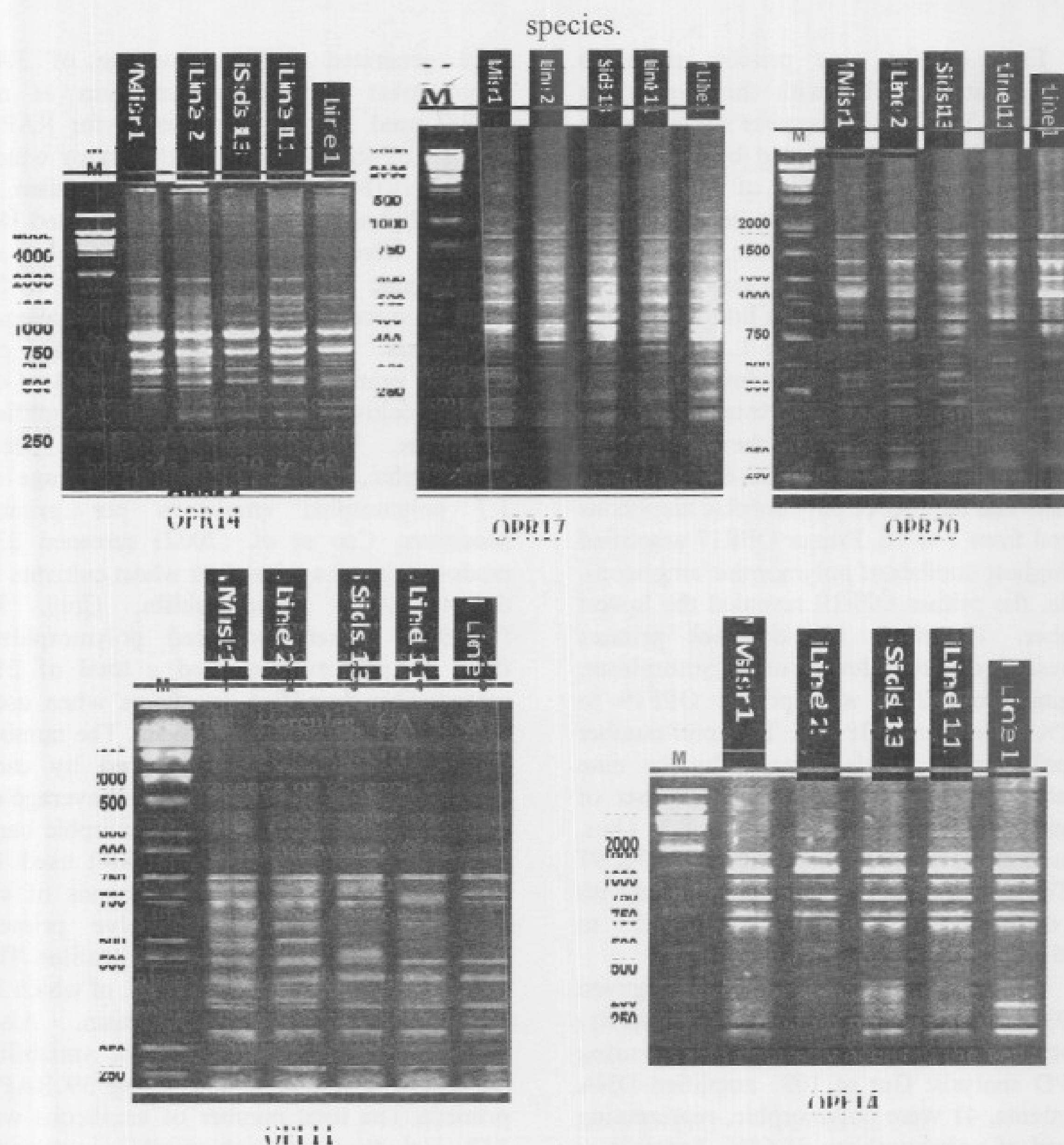

Fig.(2): RAPD profile of the five wheat genotypes (Misr1, Line 2, Sids 13, Line11 and Line 1) amplified with RAPD primers,OPR14,OPR17, OPR20, OPF11 and OPF14 : MW : 100 bp ladder.

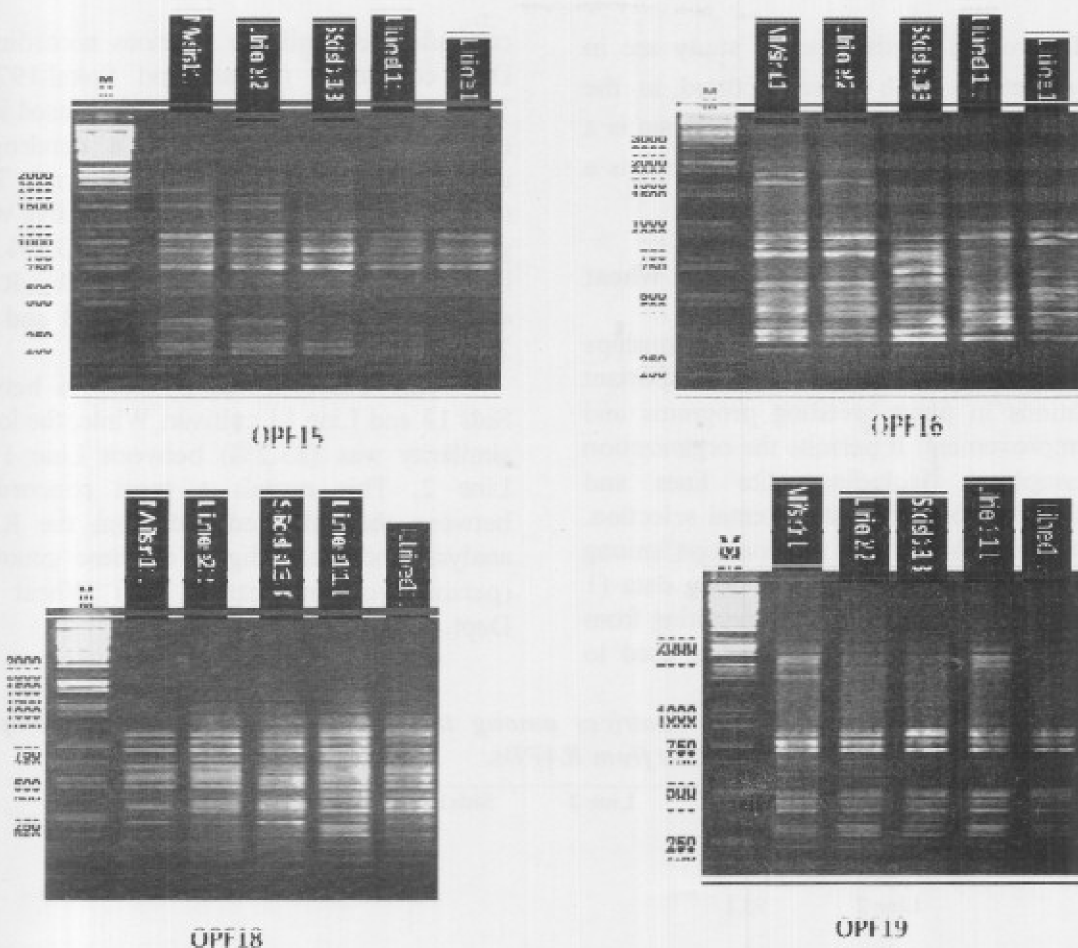

Fig. (3): RAPD profile of the five wheat genotypes (Misr1, Line 2, Sids 13, Line11,Line 1) amplified with RAPD primers,OPF15, OPF16, OPF18 and OPF19 : MW : 100 bp ladder.

Table (3): Total number of amplicons, number of monomorphic and polymorphicamplicons and percentage of polymorphism, as revealed by RAPD primers.

Primer	Total # of amplicons	#of mono amplicons	# of Poly amplicons	Polymorphism (%)
OPR14	15	5	10	66.6
OPR17	19	3	16	84.2
OPR20	15	3	12	80.0
OPF11	11	3	8	72.7
OPF14	6	2	4	66.6
OPF15	5	2	3	60.0
OPF16	12	3	9	75.0
OPF18	6	3	3	50.0
OPF19	4	3	1	25.0
Total	93	27	66	
Average	10.33	3.0	7.3	70.97

The results of the present study are in good agreement with those reported in the literature, and confirm that polymorphism is a general phenomenon in wheat although it is a self- fertilizing plant.

Genetic relationships among wheat genotypes

Knowledge of the genetic relationships among genotypes has several important applications in plant breeding programs and plant improvement. It permits the organization of germplasm, including elite lines and provides for more efficient parental selection. To determine the genetic relationships among the five wheat genotypes, the scoring data (1 for presence and 0 for absence) resulting from the nine tested RAPD primers were used to compute the similarity matrices according to Dice coefficient (Sneath and Sokal,1973) . These similarity matrices were then used in the cluster analysis to generate a dendrogram using the UPGMA method. As shown in Table (4) the genetic similarity among the five wheat genotypes ranged from 85.2 % to 93.1%. The highest genetic similarity revealed by RAPD analysis (93.1%) was between Misr1 and Line 2 genotype.

This was followed by 91.9 % between Sids 13 and Line 11 cultivar. While, the lowest similarity was (85.2%) between Line 1 and Line 2. This reveals a great concordance between the data deduced from the RAPD analysis and the pedigree of these genotypes (personal communication with Wheat Res. Dept. of ARC , Egypt)

Table (4): Genetic similarity (GS) matrices among the five wheat genotypes as computed according to Dice coefficient from RAPDs.

	Misr1	Line 2	Sids13	Line 11	Line 1
Misr1					
Line 2	93.1				
Sids13	92.6	90.7			
Line 11	90.9	91.0	91.9		
Line 1	87.5	85.2	86.5	88.4	

Cluster analysis as revealed by RAPDs

The Dice RAPD –based coefficients of genetic similarity among the 6 wheat genotypes were employed to develop a dendrogram using the UPGMA method (Fig.4).The dendrogram separated Line1 from all the other genotypes, thus demonstrating the distinctiveness of the genetic background of this genotype from all the other genotypes. The four genotypes constituted a subcluster divided into two groups, one group composed of Misr 1 and Line 2, while the second group comprised Sids 13 and Line 11. Thus, the dendrogram deduced from the RAPD data corresponded well with the pedigree of the studied wheat genotypes. The results of the present study revealed, therefore that RAPD analysis is an effective tool for detecting polymorphism, distinguishing between wheat genotypes and assessing their phylogenetic relationships. These results agree with Joshi and Nguyen (1993) who found that analysis of

the genetic relationships among wheat varieties could distinguish most of the spring and winter wheat cultivars into different clusters in the dendrogram. Sivolap *et al.* (1999) reported that RAPD analysis proved to be one of the most powerful methods of discriminating cultivars. The dendrograms based on RAPD markers most closely conform to the pedigree data. Cao *et al.* (2000) used RAPD marker to assess phylogenetic relationships between 15 wheat accessions. Cluster analysis classified these accessions into five groups in agreement with morphological classification. Sun *et al.* (2003) found that the dendrogram prepared on the basis of RAPD data corresponded well with the pedigree of two groups of wheat genotypes. Shehata *et al.*(2004) reported that SDS-PAGE and RAPD-PCR were successfully used to construct dendrograms to rate the wheat cultivars into two main groups.

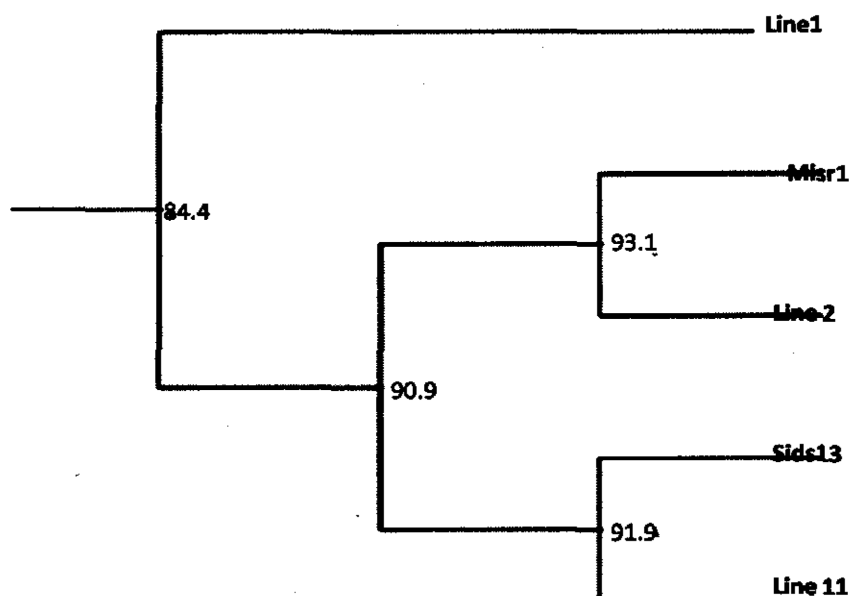

Fig. (4):Dendrogram for the five wheat genotypes constructed from RAPD data using (UPGMA) according to Dice coefficients.

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التوصيف الجزيئي لبعض التراكيب الوراثية المصرية من قمح الخبز

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كان الهدف الرئيسي من هذه الدراسه هو تقييم التباعد الوراثي بين خمسة تراكيب وراثيه من قمح الخبز (صنفي مصر 1 و سدس 13 و السلالات المبشره ارقام 1 و 2 و 11) باستخدام التفريد الكهربي للبروتين و تحليل ال RAPD .كان العدد الكلي لحزم البروتين 7 حزم. كانت 6 حزم منهم متشابهه و حزمه واحده مختلفه في السلاله رقم 1 و هي بوزن جزيئي قدره 41,56 كيلو دالتون. اظهر تحليل ال .RAPD ان عدد الحزم المختلفه كان 66 من اجمالي 93 حزمه بنسبة اختلاف قدرها 70.97%. ظهر اعلي تشابه وراثي (93,1%) بين الصنف مصر1 و السلاله رقم 2 بينما ظهر اقل تشابه وراثي (85,2%) بين السلاله رقم 1 و السلاله رقم 2 الشجره التطوريه عزلت السلاله رقم 1عن باقي التراكيب الوراثيه و قسمت الاربعة تراكيب الوراثيه الي تحت مجموعتين, تحت المجموعه الاولي تضم الصنف مصر 1 و السلاله رقم 2 بينما تشمل تحت المجموعه الثانيه الصنف سدس 13 و السلاله رقم 11.