Phylogenetic Diversity and Relationships of Some Tomato Varieties by Electrophoretic Protein and RAPD analysis

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Abstract: Biochemical and molecular characterization of eight tomato varieties were carried out based on seed storage proteins electrophoresis and RAPD markers. The electrophoretic pattern of water soluble protein produced 4 monomorphic bands, 6 polymorphic band and 3 unique bands .The pattern of non soluble protein produced 9 bands, one band is unique and considered a positive specific band of tomaten cartago variety and the others are polymorphic bands. RAPD results revealed a high level of polymorphism among the studied genotypes. All of the seven randome primers screened gave reproducible polymorphic DNA bands. A total number of 81 amplified DNA bands were generated across the studied genotypes with average of 11.57 bands /primer. 37 bands out of the total number were polymorphic and 19 were unique. Combination of the all data derived from the SDS-protein markers of both water soluble and non soluble proteins produced a dendrogram almost similar to that obtained by the RAPD analysis. It could be concluded that, both of SDS-Protein and RAPD markers are equally important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of *Lycopersicon esculentum* L.
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1. Introduction:

Tomato (*Lycopersicon esculentum* L.) is a member of the family Solanaceae and significant vegetable crop of special economic importance in the horticultural industry worldwide (He *et al.*, 2003 and Wang *et. al.*, 2005). Although the genus *Lycopersicon* includes a few species, its taxonomy is still questionable and phylogeny has not been completely established (Warnock, 1988)

The classification between various subgenera, species and subspecies is based primarily on morphological attributes. However, these morphological characters may be unstable and influenced by environmental conditions (Goodrich *et al.*, 1985). Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular trait. Therefore, the advent of the electrophoresis as an analytical tool provides indirect methods for genome probing by exposing structural variations of enzymes or other protein genome (Cook, 1984 and Gilliand, 1989).

The electrophoresis of proteins is a method to investigate genetic variation and to classify plant varieties (Isemura *et al*., 2001). Its banding pattern is very stable which advocated for cultivars identification purpose in crops. It has been widely suggested that such banding patterns could be important supplemental method for cultivars identification (Tanksley and Jones, 1981; and Thanh and Hirata, 2002). Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding.

DNA molecular markers technology, which are based on sequence variation of specific genomic regions, provide powerful tools for cultivar identification and seed quality control in various crops with the advantages of time-saving, less labor-consumption and more efficiency (Hu and Quiros, 1991; Mongkolporn *et al*., 2004; Dongre and Parkhi, 2005; Garg *et al*., 2006 and Liu *et al*., 2007).

Random Amplified Polymorphic DNA (RAPD) is based on *in vitro* amplification of randomly selected oligonucleotide sequences. Amplification takes place by simultaneous primer extension of complementary strands of DNA; the primers use the plant DNA as a template for PCR amplification. RAPD is very useful in the study of biodiversity, hybridization, gene mapping and genetic map construction (Sharma and Sharma, 1999).

Generally, molecular markers have proven to be useful tools for characterizing genetic diversity in agricultural crops. Researchers have studied genetic variation in tomato landrace and cultivar collections using various molecular techniques, including restricted fragment length polymorphism

(RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) (Miller and Tanksley, 1990, Rus- Kortekaas *et al*., 1994, Bredemeijer *et al*., 1998, Villand *et al*., 1998, Mazzucato *et al*., 2003, Park *et al*., 2004, Carelli *et al*., 2006 Garcia-Martinez *et al*., 2006) . The aim of the present study was to find out the phylogenetic relationships of eight tomato varieties using protein profiles and random amplified polymorphic DNA (RAPD) analysis.

2. Materials and methods

Seeds of eight tomato cultivars of diverse origins grown in Egypt (Tomaten cartago GC781, Karnak, Fac-68, Floradid, Jack pot, Casel rock, Pack-mor and Petto 86) were used for the present Study and kindly supplied by Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

SDS-protein electrophoresis:

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for water soluble and non soluble proteins was performed according to the method of Laemmli (1970), as modified by Studier (1973). Molecular weights of different bands were calibrated with a mixture of standard protein markers include myosin (212 KDa), B-galactosidase (120KDa), phosphorylase b (97KDa), bovinserumalbumin (66.2KDa), ovalbumin (45KDa), carbonic anhydrase (31KDa), soybean trypsin inhibitor (20KDa), lysozyme (14.4KDa) and aprotinin (6.5KDa) from Bio Basic Inc. The banding profile was photographed and scored.

DNA isolation:

Genomic DNA was isolated from the young leaves by using Bio Basic Kits. The quality of isolated genome DNA was checked by agarose gel electrophoresis.

DNA amplification:

Seven decamer oligonucleotide primers obtained from (metabion international AG) were used for the polymorphism survey. Amplification reactions were carried out in 25µL volumes, containing (5 µL of 5x buffer, 3.0 µL of dNTPs (2.5mM) 3 µL of Mg cl2 (25 m M), 3.0 µL primer (2.5 µ L) ,0.3 µ L of Taq polymerase(5U/ µL) and 2.0 µL of genomic DNA (50 ng/ µL). Amplification was performed in PTC-100 PCR version 9.0 from M J Research-USA. Programmed for an initial denaturation at 94 ^{o}C 5 min, 40 cycles of 1 min denaturation at 94 ^{o}C, 1 min annealing at 40^{o}C and 2 min extension at 72^{o}C followed by final extension for 5 min at 72^{o}C.

Amplified products from the RAPD reactions were separated by horizontal gel electrophoresis unit using 1.5% agarose gel in TAE buffer and stained with ethidium bromide .The run was performed at 95 volt for 55 min. DNA ladder 1Kb was used from fermentas with lengths ranged from 264 to 11507 bp and then the gel was visualized by UV-transilluminator to examine the reproducibility of banding patterns, then photographed by gel documentation system, Biometra – Bio Doc Analyze. Each PCR reaction was repeated twice in order to ensure that RAPD banding patterns were consistent and reproducible and only stable products were scored.

Statistical analysis:

The electrophoretic patterns of water soluble and non soluble proteins, and the reproducible banding patterns of each primer which produced by RAPD were chosen for analysis. Each gel was scored as present (1) or absent (0), and pair wise comparisons between individuals were made to calculate the Jaccard's coefficient of genetic similarity matrix using SPSS program (statistical Package for Social Scientists) version-10 (Norman *et al.*, 1975). Cluster analysis was performed to produce a denderogram using unweighted pair-group method with arithmetical average (UPGMA).

3. Results and Discussion:

1. SDS protein analysis

1-1. Water soluble protein:

SDS-electrophoretic patterns of water soluble and water non soluble protein fractions were used to find out the phylogenetic relationships among some varieties of tomato. Figure (1) demonstrates the water soluble protein banding patterns of the eight tomato varieties belonging to (*Lycopersicon esculentum* L.).

The SDS banding pattern of water soluble protein produced 13 bands distributed in all varieties with molecular weights ranging from 6.50 KDa to 130.24 KDa. with polymorphism percentage reached to 86.231% between the eight tomato varieties. The pattern of these bands is as follows, 4 bands are monomorphic with molecular weights of 42.55, 18.14, 11.08 and 9.63 KDa., while 6 bands are Polymorphic with molecular weights of 103.09, 46.89, 26.67, 12.62, 8.31 and 7.15 KDa., in addition to three unique bands which have been observed in three varieties, Jack Pot had one of these positive specific band with molecular weight of 130.24 KDa. Also, Casel Rock had one positive specific band with molecular weight of 16.33 KDa, and tomaten Cartago had the third positive specific bands at molecular weight of 6.50 KDa. These bands could be

considered as specific markers for distinguishing these varieties from each others.

1-2. Water non soluble protein:

The SDS banding pattern of water non soluble protein produced 9 bands distributed in all varieties with molecular weights ranging from 6.50 to 69.02 KDa. The polymorphism percentage reached to 100% between the studied tomato varieties. Figure (1) shows that water non soluble protein bands distributed a follows, only one band is unique with molecular weight of 14.37KDa. and considered a positive specific band of tomaten cartago variety, while the others are polymorphic bands.

The UPGMA method was used to calculate the similarity coefficient among the studied tomato varieties of both water soluble and water non soluble proteins individually, based on existence of the bands (presence or absence) and their average was used as an approximate value for recognizing groups of varieties in dendrogram (Fig.2 a&b), which showed the same relationship between tomato varieties, when the data of water soluble proteins and water non soluble proteins were combined for UPGMA cluster analysis, the obtained dendrogram (Fig.2c) revealed almost the same cluster pattern of the eight studied tomato varieties.

The similarity coefficient based on water soluble and non soluble proteins markers ranged from 0.771 to 0.135. Table (1) showed that the highest similarity value (0.771) was between Cartago and Jac-pot. While the lowest value of (0.135) was between Cartago and Betto. The relationship between Casel rock and Floradid was 0.574, and between Casel rock and Fac-68 was 0.646. Betto is equally closely related to both Karnak and Pack-mor (0.733).

The clusters obtained from the dendrogram Fig. (2c). showed that the studied tomato varieties are grouped in three main groups.The first one consists of tomaten cartago and jack-pot which were the most related varieties to each other. The second group includes Casel rock, Floradid and Fac-68 .while, the third groupe consists of karnak , Betto and Pack-more ,therefore , it could be concluded that the results of water soluble and non soluble protein could differentiate between the studied tomato varieties producing some specific bands that can be used to distinguish such variety from each others. These specific variations were analyzed to assess the protein polymorphisms between different varieties of tomato and clarify the genetic nature of polymorphic bands. Similary, different cultivars of cultivated chickpea were examined by Ahmad and Slinkard (1992), they concluded that seed protein was a very conservative trait in chickpea. Also, Raymond *et al.* (1991), and De vries (1996), reported similar electrophoretic patterns of protein among the cultivars of sunflower and lettuce. Munazza *et al.* (2009) studied the electrophoretic characterization in different genotypes of oilseed Brassica based on analysis of seed storage proteins to assess the protein polymorphisms within and different cultivated species and clarify the genetic nature of polymorphic bands to differentiate the yellow and brown seeded varieties of Brassica.

Furthermore, This electrophoretical proteins can detect genetic purity test in case of vegetables such as tomato by several studies using isozyme and protein polymorphism (Thanth *et al*., 2006, Tanksley and Jones, 1981; Wang *et al*., 2005).The reduction of genetic variation in tomato (Solanum lycopersicum L.) through domestication and breeding (Tanksley and McCouch,1997; Barrero and Tanksley,2004) has resulted in the need for conservation, characterization, and utilization of genetic resources.

The high stability of protein profile makes protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants (Ladizinsky and Hymowitz,1979), so it seems to say that SDS-PAGE technique has proven to be a useful in supporting classical taxonomy studies (Thanh *et al.*, 2003).

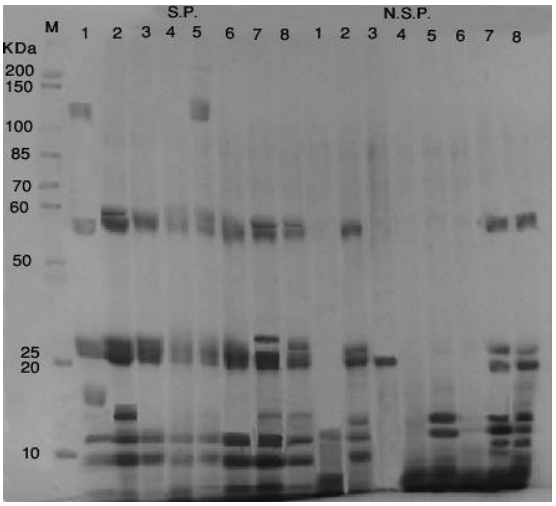

Fig. (1) SDS-protein banding pattern of water soluble and water non soluble proteins of the eight tomato genotypes. 1. cartago GC781 2. Karnak 3. Fac-68 4. Floradid 5. Jack pot 6.Casel rock 7. Pack-mor 8.Petto 86.

Table (1): Similarity coefficients of the eight tomato varieties based on water soluble and non soluble proteins markers.

Case	Matrix file input							
Car.	1.00							
Kar.	0.142	1.00						
Fac.	0.443	0.541	1.00					
Flo.	0.357	0.357	0.746	1.00				
Jac.	**0.771**	0.255	0.357	0.341	1.00			
Cas.	0.535	0.357	**0.646**	**0.574**	**0.357**	1.00		
Pac.	0.142	**0.711**	0.355	0.257	0.264	0.243	1.00	
Bett.	0.135	**0.733**	0.341	0.357	0.205	0.443	**0.733**	1.00
	Car.	Kar.	Fac.	Flo.	Jac.	Cas.	Pac.	Bett.

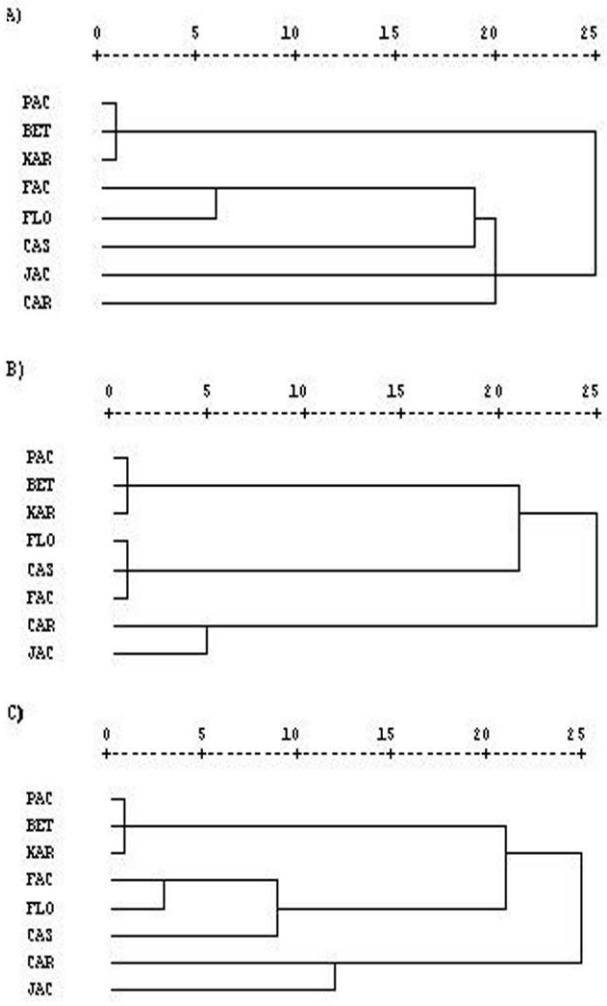

Fig. (2): UPGMA dendrogram indicating the genetic relationships among the eight tomato varieties based on:

a) water soluble proteins markers
b) water non soluble proteins markers
c) combination of water soluble and non soluble proteins

2- Molecular studies:

Seven RAPD primers were tested against the eight tomato varieties. The sequences of these primers are listed in Table (2). The RAPD profiles of the amplified products are shown in Fig. (3).The number of bands and the degree of polymorphism revealed by each primer are given in Table (2). Generally, the levels of polymorphism were varied with different primers among the different tomato varieties. The percentage of polymorphism produced by each primer differed from one primer to the other, the maximum value of polymorphism was 85.714% produced by primer OPD-13. While, the minimum value of polymorphism was 45.455% by primer OPX-17, with an average polymorphism of 66.479 % across all the genotypes.

A total number of 81 amplified DNA bands were generated across the studied genotypes with average of 11.57 bands/ primer. Out of the total band, 37 polymorphic and 19 unique ones were detected. The RAPD profiles of the amplified products of each primer are shown in Fig.3 (a, b, c, d, e, f and g). A maximum number of 17 bands were amplified with primer opc-19 and a minimum of 7 bands with primer OPD-13. The number of monomorphic bands was primer dependent and ranged from 1 band by primer D-13 to 6 bands by primer OPN-06 and primer OPX-17.

The genetic similarity coefficient, recognized the eight studied tomato variety, the highest similarity value was 0.891 which recorded between Cartago and Jac-pot, while the lowest similarity value was 0.401 between cartago and Karnak Table (3).

Similarity coefficient matrices were used to generate a dendrogram of tomato genotypes based on UPGMA analysis Fig (4), the analysis divided the eight genotypes into three distinct clusters .The first cluster includes Cartago and Jac-Pot with the highest similarity value (0.891), while the second cluster contains three genotypes, Floradid and Casel Rock which are moderately related (0.673) then come Fac-

68 which is highly related to Casel Rock (0.810). The third cluster comprises of Betto, Pack-more and Karnak. In which Pack-mor and Karnak are highly related to each other (0.864) and Betto was closer to Pack-more (0.795) than Karnak (0.699).

From the previous results it could be concluded that, the dendrogram on the basis of RAPD revealed almost the same phylogenetic relationships between the eight studied tomato varieties that obtained by combining the data from the markers of water soluble and non soluble proteins.

Some earlier researchers stated that the application of both biochemical and molecular genetics techniques have an important potential to provide a new tool for the study of both wild and domesticated species in respect to investigation of evolution and migration of species from their gene pool centers (Badr *et al*., 2000 and Fregonezi *et al* ., 2006).

The identification and characterization of species become possible through fingerprinting for each species since DNA is a source of informative polymorphism (El-Rabey, 2008), consequently, techniques of molecular genetic markers have an important potential for the detection of genetic differences among species (Benmoussa and Achouch, 2005). Many investigations reported that, RAPD analysis is revealed high genetic polymorphism of the tomato genome and established the phylogenetic relationships among members of the genus *Lycopersicon* Mill. The resulting dendrogram was consistent with *Lycopersicon* phylogeny based on the molecular data of RFLP, ISSR, microsatellite analysis and with the classification based on morphological characters (Ruck, 1979; Palmer and Zamir, 1982; Miller and Tanksley 1990; Khrapalova, 1999 and Lingxia *et al*., 2009). Therefore, the use of molecular markers in the applied breeding programs can facilitate appropriate choice of parents involved for crosses. Munazza *et al*., (2009) reported that the assessment of genetic diversity within and between landraces should have priority for varieties improvement. At the same time it is necessary to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation and collection of germplasm and to increase the utilization of plant genetic resources.

4. Conclusion:

Eight tomato varieties were used in order to elucidate their genetic diversity by using SDS-proteins and RAPD-PCR analysis.

It could be concluded that the present biochemical results, water soluble and non soluble protein, can differentiate between the studied tomato varieties by producing some specific bands that could be used to distinguish any variety from each others. These specific variations were analyzed to assess the protein polymorphisms between different varieties of tomato and clarify the genetic nature of polymorphic bands. On the molecular level, seven primers were used to differentiate between these varieties and gave reproducible results with wide variations in their band numbers. The molecular markers obtained by the RAPD technique revealed a remarkable molecular discrimination between the eight tomato varieties under the study. The phylogenetic analysis on the basis of RAPD derived a dendrogram revealed almost the same cluster pattern that obtained from the combined markers of water soluble and non soluble proteins and confirm the phylogenetic relationship between the eight studied tomato varieties. It could be concluded that, both of SDS-Protein and RAPD markers are equally important for genetic analysis and indicate a considerable amount of genetic diversity between the different studied varieties of *Lycopersicon esculentum* L.

Table (2): Code and sequence of the seven DNA random primers used for identifying the tomato varieties and types of the amplified DNA bands.

Primers Cod	Sequence	Total No. of bands	No. of Monomorphic bands	unique band	No. of polymorphic bands	% of Polymorphic loci.
OPA-03	5'-AGTCAGCCAC-3'	13	2	6	5	84.615
OPC-19	5'-GTTGCCAGCC-3'	17	3	4	10	82.353
OPD-13	5'-GGGGTGACGA-3'	7	1	3	3	85.714
OPN-06	5'-GAGACGCACA-3'	8	6	1	1	25.000
OPT-08	5'-AACGGCGACA-3'	11	4	1	6	63.636
OPW-04	5'-CAGAAGCGGA-3'	14	3	2	9	78.571
OPX-17	5'-GACACGGACC-3'	11	6	2	3	45.455
Total	-	81	25	19	37	-
Average/ primer	-	11.57	-	-	-	66.479%

Table (3): Similarity coefficients of the eight tomato varieties based on RAPD markers.

Matrix File Input

Case								
Car.	1.000							
Kar.	0.401	1.000						
Fac.	0.612	0.482	1.000					
Flo.	0.686	0.517	0.673	1.000				
Jac.	0.891	0.545	0.591	0.545	1.000			
Cas.	0.479	0.673	0.810	0.673	0.509	1.000		
Pac.	0.455	0.864	0.533	0.427	0.445	0.471	1.000	
Bett.	0.469	0.699	0.682	0.582	0.500	0.536	0.795	1.000
	Car.	Kar.	Fac.	Flo.	Jac.	Cas.	Pac.	Bett.

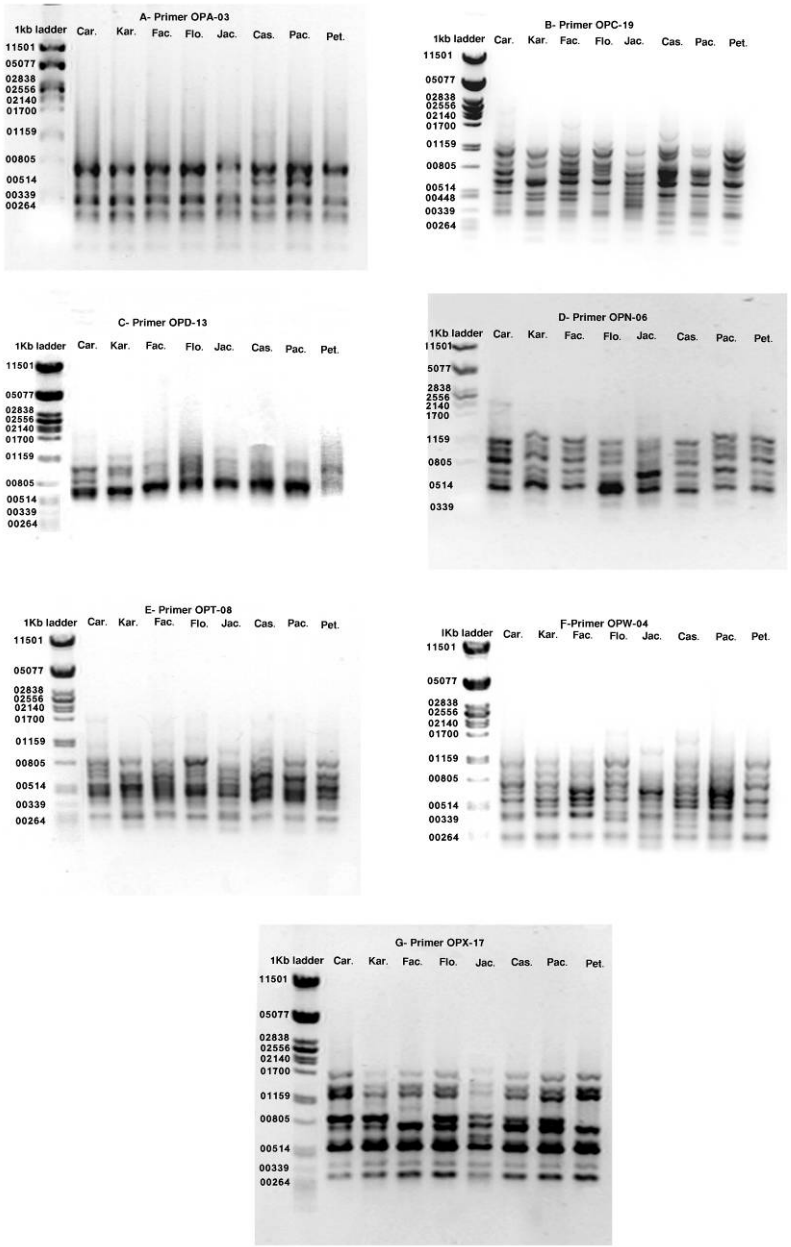

Fig. (3): RAPD fingerprints of the eight obtained tomato varieties generated by the seven primers a) OPA-03 b) OPC-19 c) OPD-13 d) OPN-06 e) OPT-08 f) OPW-04 g) OPX-17

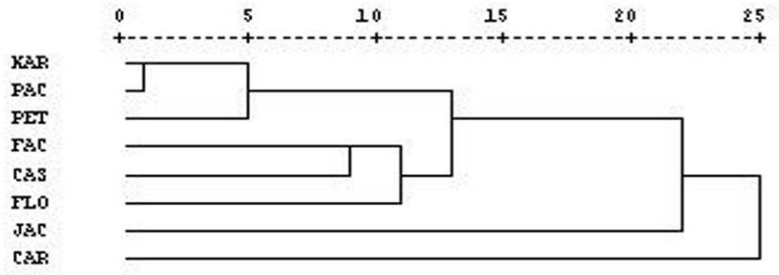

Fig (4): UPGMA dendrogram indicating the genetic relationships among the eight tomato varieties based on RAPD markers.

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