ASSESSING GENETIC DIVERSITY OF SOME POTATO (*Solanum tuberosum* L.) CULTIVARS BY PROTEIN AND RAPD MARKERS

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Potato (*Solanum tuberosum* L.) is one of the most important crops grown in Egypt for local consumption, export and processing industries. The area cultivated with potatoes represents about 212,000 acres producing 2.2 million tons, with an average of 10.5 tons per acre (Abd-Elgawad and Youssef, 2008). Furthermore, it is a good source of vitamins, mainly B and C complexes, and mineral salts. The success of potato breeding programs depends on identification of the amount and distribution of genetic diversity in the gene pool, to identify the gaps in germplasm collections and to develop effective conservation and management strategies (Esfahani *et al*., 2009). Traditionally, morphological characters are used for establishing the identity of cultivars but these characters are under the influence of environmental changes, epistatic interactions and pleiotropic effects. Biochemical and molecular analysis make it possible to establish differences at various taxonomic levels which in turns help the researchers to assess genetic diversity in the investigated germplasm (Rabbani *et al*., 2001; Pervaiz *et al*., 2010; Mumtaz *et al*., 2010). One of the biochemical methods extensively used in taxonomic and assessment of genetic diversity studies is the electrophoretic analysis of the proteins using Sodium Dodecylsulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). These proteins are physiologically stable and easy to handle (Ladizinsky and Hymowitz, 1979). They operate at the level of gene product where the environment has very little influence (Feldman and Sears, 1981). Furthermore, DNA fingerprinting techniques have been developed for measuring genetic variability and cultivar identification. The most common techniques include PCR-based assays such as Randomly Amplified Polymorphic DNA (RAPD; Williams *et al*., 1990), Simple Sequence Repeats (SSR; Akkaya *et al*., 1992), Amplified Fragment Length Polymorphism (AFLP; Vos *et al*., 1995) and Inter Simple Sequence Repeats (ISSR; Zietkiewicz *et al*., 1994).

Randomly Amplified Polymorphic DNA (RAPD) is a very useful tool in the study of biodiversity, hybridization, gene mapping and genetic map construction (Sharma and Sharma, 1999). RAPD technique is being used successfully to identify, characterize and estimate genetic divergence of potato cultivars (Isenegger *et al*., 2001; Miller and Spooner, 1999; Moisan *et al*., 2001; Spooner *et al*., 2005; Sun *et al*., 2003; Rocha *et al*., 2010). Iden-

tification of genetic diversity using DNA markers in potato can provide insights into the genetic structure and diversity among varieties from different geographical origins. When the magnitude and nature of genetic diversity is estimated in advance, a suitable selection strategy is planned according to heritability of genetic traits (Ghislain *et al*., 1999). A combination of passport data and genetic diversity information from molecular markers would therefore enhance the formation of germplasm stocks and could be useful tool in the calculation of genetic distance of the potato genotypes (Akkale *et al*., 2010). The aims of this study were to evaluate the genetic diversity in nine potato cultivars that are being made available to potato growers in Egypt, and to develop a molecular profile using RAPD and protein markers for cultivars identification purposes.

MATERIALS AND METHODS

Certified seed tubers of potato (*Solanum tuberosum* L.) cvs, Cara, Spunta Nais, Maranca, Draga, Aramada, Hana, Diamant, Spunta and Spunta Hema were kindely provided by AGRICO, company. The tubers were placed in dark at 20°C to sprouting, sprouted tubers were planted in a sterile mixture of sand-vermiculite and peat (2:2:1) and grown at 25°C.

SDS-protein electrophoresis

Protein extracts were prepared from young leaves (~0.5 g) and homogenates were obtained by mechanically grinding in 500 µl of the protein extraction buffer (62.5 mM Tris-Hcl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 5 M Urea and 0.01% bromo-phenol blue) was added and mixed well by vortexing. Protein extracts were centrifuged at 14,000 rpm for 10 min at 4°C and apply in 12% (SDS-PAGE) according to (Laemmli, 1970). Molecular weights of different bands were calibrated with a mixture of standard protein markers (Molecular Weight Marker, M. W. 14.000-66.000; Catalog No. SDS7). The banding profile was stained by Coomase blue dye then photographed and scored.

Molecular analysis

DNA was extracted from fresh leaves by Cetyltrimethyl Ammonium Bromide (CTAB) according to (Doyle and Doyle, 1990). RAPD was performed using 24 random decamer primers (Table 1). Polymerase Chain Reaction (PCR) was carried out in presence of 1X Taq DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 100 µM dNTPs, 5 picomole single random primer, 25 ng template DNA, 0.5 unit of Taq DNA polymerase in a total volume of 25 µl. PCR amplification was performed in automated thermal cycler (MJ-Mini, Bio Rad) programmed as follow, 95°C for 4 min followed by 40 cycles of 1 min for denaturation at 94°C, 30 sec for annealing at 37°C and 1.30 min for polymerization at 72°C, followed by a final extension step at 72°C for 7 min. The amplification products were resolved by electrophoresis in 1.5% agarose gels in 0.5 X TBE buffer using O'GeneRuler™ 100 bp Plus DNA

Ladder, gels were documented on Gel Documentation UVITEC, UK.

Data analysis

The electrophoretic patterns of 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 PAST program (PAleontological Statistics Version 1.94b, Hammer *et al.*, 2001). Cluster analysis was performed to produce a denderogram using unweighted pair-group method with arithmetical average (UPGMA).

RESULTS AND DISCUSSION

Protein analysis

The SDS protein banding patterns produced 28 bands distributed in all cultivars with molecular weights ranging from 21.30 KDa to 112.6 KDa. Fourteen out of 28 bands were polymorphic (50% polymorphism). All nine potato cultivars were clearly identifiable from protein banding pattern (Fig. 1). Protein patterns showed that almost of bands were differ in their intensity among studied cultivars. Moreover, some cultivars possessed some bands which were absent in other cultivars. Band No. 4 (p4; 98.4 KDa) was found only in Maranca and Armada, moreover, band No. 5 (p5; 79.1 KDa) was unique for Maranca. Contrary, bands No. 7 (p7; 66.9 KDa) and No. 14 (p14; 47.1 KDa) were found in all studied cultivars and absent in the Maranca and Drage, respectively. The potato cultivars Draga and Maranca comparatively showed more divergence than other potato cultivars. The similarity coefficient based on protein pattern ranged from 0.903 to 0.565. The highest similarity value (0.892) was between Diamant and Hana, while the lowest value of (0.565) was between Draga and Maranca, while the similarity between Spunta Hema and Armada was 0.809 (Table 2). The UPGMA cluster analysis revealed that the nine potato cultivars could be divided into three major clusters, the first one includes the highly diverged cultivar (Draga), the second cluster contains Maranca cultivar, while the third cluster divided into two groups; one of them contains Armada and Spunta Hema whereas the second group encircled cultivars having high degree of similarity (Fig. 3). A comprehensive analysis of the extent and distribution of the genetic variation in potato is essential for sound genetic conservation strategies. Conservation and sustainable use of genetic resources is essential to meet the demand for future food security. Successful conservation of any given gene pool is largely depends on understanding the diversity and its distribution in a given region (Zhang *et al*., 2000). In this study, high level of genetic diversity was exhibited in potato. These specific variations were analyzed to assess the protein polymorphisms among different cultivars of potato and clarify the genetic diversity. Similarly, different cultivars of cultivated chickpea were examined by Ahmad and Slinkard (1992); they concluded that seed

protein was a very conservative trait in chickpea. 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* Munazza *et al.* (2009). Furthermore, these 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; Wang *et al.*, 2005). It seems to say that SDS-PAGE technique has proven to be a useful in supporting classical taxonomy studies (Thanh *et al.*, 2003; Abd El-Hady *et al.*, 2010).

Protein markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Gepts *et al.*, 1986). Protein markers have been used to study crops including *Solanum* spp. (Mennella *et al.*, 1999). Total protein markers are also used extensively in cultivars characterization (Mollema and Cole, 1996). One major importance of the integration of markers to study diversity is that it allows for better discrimination among accessions than a single method. However, although protein electrophoresis provides a means of estimating levels of genetic variation, much variation may still remain undetected at the level as only parts of the genome which are expressed can be detected. Therefore, it could be concluded that the results of protein could differentiate between the studied potato cultivars producing some specific bands that could be used to distinguish such variety from each other.

Molecular analysis

Twenty four RAPD primers were tested against the nine potato cultivars. The sequences of these primers, the number of bands and the degree of polymorphism revealed by each primer (Table 1). The RAPD profiles of the amplified products are shown in Fig. (2). Different banding patterns were obtained from different primers used (Fig. 2). A total number of 308 amplified DNA bands were generated across all cultivars with average of 12.8 bands per primer. Generally, the levels of polymorphism were varied with different primers among the different potato cultivars. The percentage of polymorphism produced by each primer differed from one primer to the other; the polymorphism ranged from 44.44% using primer OPA07 to 100% using primer OPK07 across all the cultivars. Primer OPK04 produced the highest number of bands (28) while, primer OPA06 amplified the lowest number of bands (4). The highest similarity value (0.892) was between Diamant and Hana, while the lowest value of (0.335) was between Draga and Maranca (Table 3). The relationship between Spunta and Armada was 0.784, and between Spunta and Hana was 0.820. Armada is equally closely related to both Spunta and Hana (~0.78).

Similarity coefficient matrices were used to generate a dendrogram of potato cultivars based on UPGMA analysis Fig. (4), the dendrogram divided the

nine potato cultivars into three distinct clusters. The first and second clusters consisted of only two potato cultivars (Draga and Maranca) that showed more divergence and individuality in their banding outline comparatively from other potato cultivars. The third cluster divided into two sub clusters; where Cara and Spunta Nais were grouped together and the second sub cluster consisted of cultivars having high degree of similarity (Hana, Diamant, Spunta and Armada). The use of a range of currently available DNA-based markers complements variability detected at both the morphological and protein levels. The results, nonetheless, provide useful information for gene bank curators. The results obtained in this work showed that RAPD analysis could provide an easy, low cost and quick way for the identification of potato cultivars and also have a better knowledge of the genetic affinity. The variation in the number of bands amplified by different primers influenced by variable factors such as primer structure, template quantity and less number of annealing sites in the genome. RAPD technique is being used successfully to identify, characterize and estimate genetic divergence of potato cultivars (Isenegger *et al*., 2001; Miller and Spooner, 1999; Moisan *et al*., 2001; Spooner *et al*., 2005; Sun *et al*., 2003).

From the aforementioned results it could be concluded that the dendrogram on the basis of RAPD revealed almost the same phylogenetic relationships between the nine studied potato cultivars that obtained by the data from the proteins marker. Furthermore, both types of marker could be used in assessing generic diversity and characterization of some potato cultivars grown in Egypt.

SUMMARY

Genetic diversity among nine potato cultivars was investigated using protein and RAPD markers. The electrophoretic pattern of protein analysis produced 28 bands distributed in all cultivars with molecular weights ranging from 21.30 KDa to 112.6 KDa. Some cultivars possessed some bands which were absent in other cultivars. Furthermore, RAPD analysis revealed a high level of polymorphism among the studied genotypes. A total number of 308 amplified bands were generated across the studied genotypes with average of 12.83 bands/primer. SDS-protein marker 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 potato.

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Table (1): Primers, their respective base sequences, number of amplified bands, number of polymorphic bands and polymorphism percentages for the nine potato cultivars analyzed.

Primer Name	Sequence (5′ → 3′)	Total No. of bands	No. of Polymorphic bands	Polymorphism %
OPA-04	AATCGGGCTG	10	7	70.00
OPA-06	GGTCCCTGAC	4	3	75.00
OPA-07	GAAACGGGTG	9	4	44.44
OPA-11	CAATCGCCGT	22	21	95.45
OPA-12	TCGGCGATAG	14	13	92.85
OPA-13	CAGCACCCAC	12	11	91.66
OPA-14	TCTGTGCTGG	15	14	93.33
OPA-16	AGCCAGCGAA	16	14	87.50
OPK-01	CATTCGAGCC	7	4	57.14
OPK-02	GTCTCCGCAA	15	10	66.66
OPK-03	CCAGCTTAGG	15	13	86.66
OPK-04	CCGCCCAAAC	28	27	96.42
OPK-05	TCTGTCGAGG	7	6	85.71
OPK-06	CACCTTTCCC	11	6	54.54
OPK-07	AGCGAGCAAG	17	17	100.00
OPK-08	GAACACTGGG	11	5	45.45
OPK-09	CCCTACCGAC	16	11	68.75
OPK-10	GTGCAACGTG	10	8	80.00
OPK-11	AATGCCCCAG	12	10	83.30
OPK-12	TGGCCCTCAC	17	13	76.47
OPK-16	GAGCGTCGAA	12	6	50.00
OPK-17	CCCAGCTGTG	9	8	88.88
OPK-18	CCTAGTCGAG	9	7	77.70
OPK-19	CACAGGCGGA	10	6	60.00

Table (2): Similarity matrix among the nine potato cultivars based on protein analysis.

	Cara	Spunta Nais	Maranca	Draga	Aramada	Hana	Diamant	Spunta
Spunta_Nais	0.849							
Maranca	0.691	0.693						
Draga	0.571	0.666	0.607					
Aramada	0.783	0.754	0.782	0.565				
Hana	0.772	0.863	0.708	0.739	0.752			
Diamant	0.750	0.795	0.686	0.684	0.732	0.903		
Spunta	0.765	0.818	0.786	0.787	0.725	0.891	0.813	
Spunta_Hema	0.796	0.747	0.722	0.588	0.809	0.803	0.800	0.757

Table (3): Similarity matrix among the nine potato cultivars based on RAPD analysis.

	Cara	Spunta Nais	Maranca	Draga	Aramada	Hana	Diamant	Spunta
Spunta_Nais	0.706							
Maranca	0.630	0.648						
Draga	0.356	0.377	0.335					
Aramada	0.705	0.675	0.610	0.391				
Hana	0.718	0.680	0.634	0.374	0.780			
Diamant	0.714	0.691	0.602	0.370	0.783	0.820		
Spunta	0.651	0.696	0.618	0.358	0.755	0.892	0.774	
Spunta_Hema	0.676	0.703	0.626	0.386	0.736	0.715	0.737	0.716

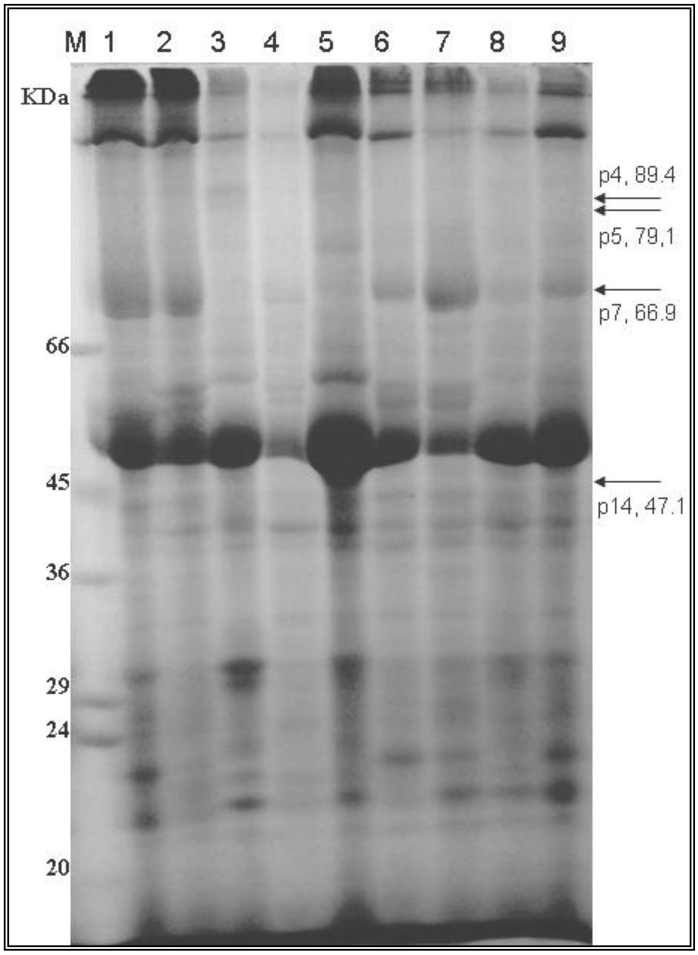

Fig. (1): Protein banding patterns of the nine potato cultivars, Lanes from: 1 to 9 represent Cara, Spunta_Nais, Maracna, Draga, Aramada, Hana, Diamant, Spunta and Spunta_Hema, M, Protein Molecular Weight Marker, M. W. 14.000-66.000 KDa.

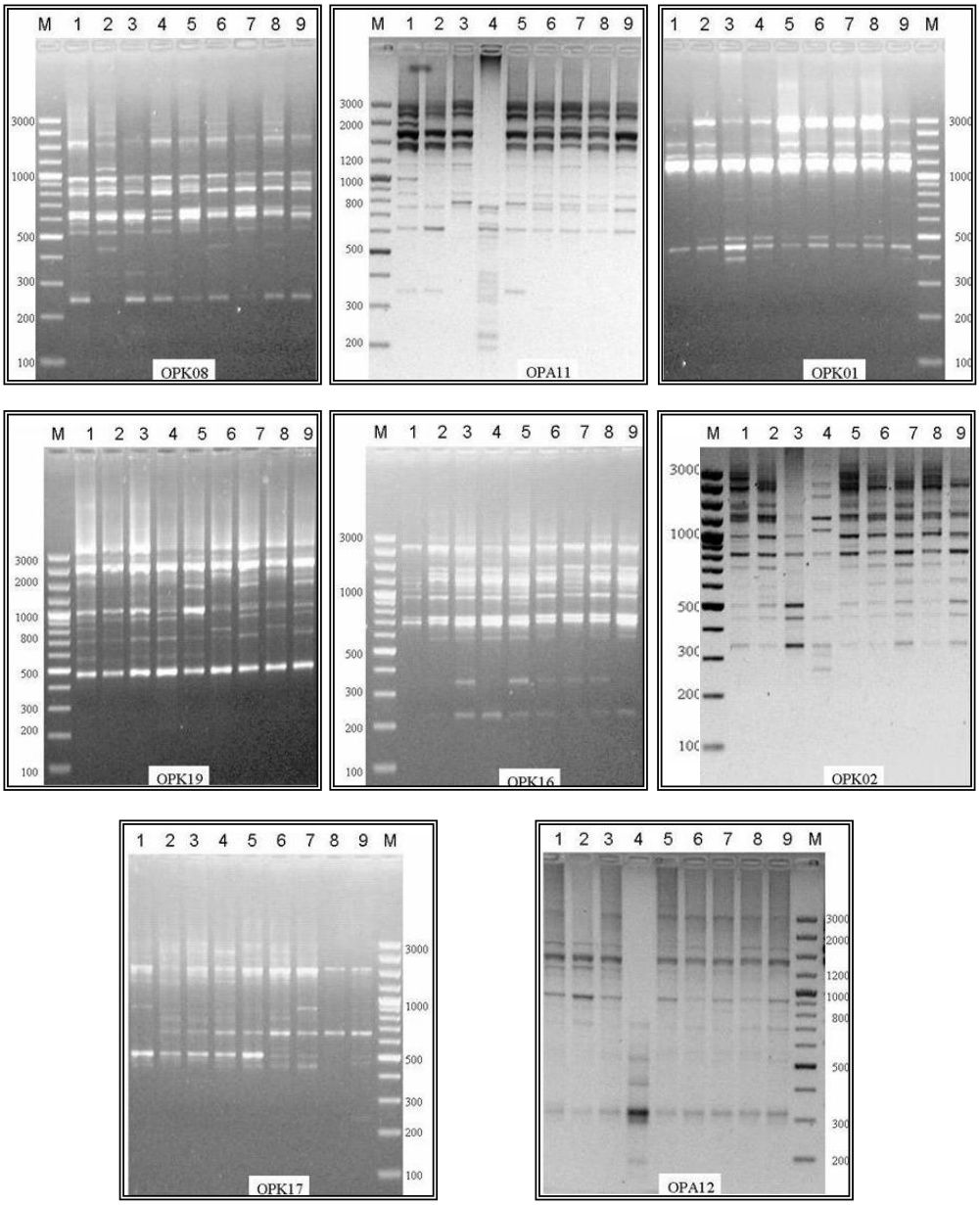

Fig. (2): RAPD profiles of the nine potato cultivars. Lanes from: 1 to 9 represents Cara, Spunta_Nais, Maranca, Draga, Aramada, Hana, Diamant, Spunta and Spunta_Hema, M, DNA marker (1 kb).

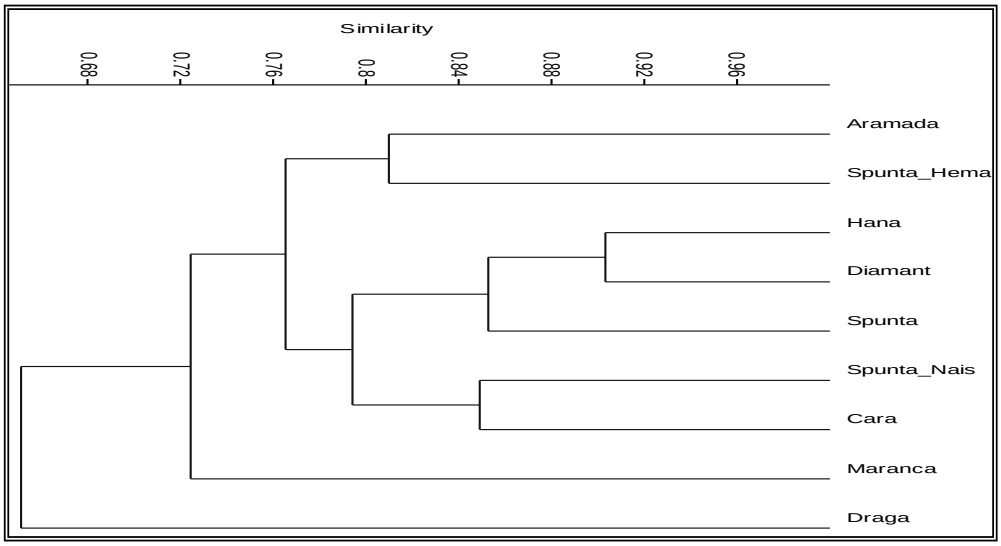

Fig. (3): Dendrogram showing genetic variability and relationships among nine potato cultivars using protein marker.

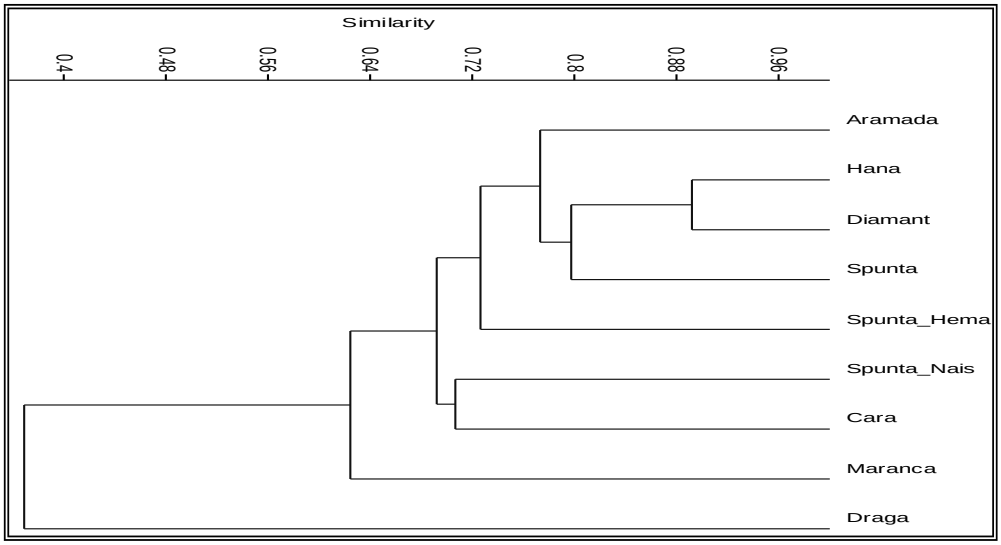

Fig. (4): RAPD based dendrograms of the nine potato cultivars constructed using unweighted-pair group arithmetic average (UPGMA) and similarity matrices.