Molecular Genetic Evaluation Of Seven Varieties Of Summer Squash

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ABSTRACT: The present investigation was carried out in 2010 growing season, with the aim of molecular genetic evaluation of seven squash parents under Egyptian conditions. The study included seven squash varieties. These selected squash parents were: i.e. Eskandrani (P_1), Zucca Patisson custard white (P_2), All Green Bush (P_3), Courgette Orelia (P_4), Sakiz (P_5), Copi (P_6) and Gapla (P_7). These parents were discriminated by their leaves fingerprints as obtained through protein electrophoresis technique and RAPD-PCR technique using five random primers. Protein electrophoresis successfully generated reproducible polymorphic banding patterns. The generated profiles revealed high levels of polymorphism among the studied parents. Data of the analysis recorded a sum of 18 bands. These bands were identified as 11 polymorphic bands and 7 monomorphic ones in all studied parents. The polymorphic bands were scored as 3 unique bands. These unique bands were used to discriminate between the seven squash parents. Five 10-mer arbitrary primers of twenty-one of each RAPD successfully generated reproducible polymorphic products. The generated profiles revealed high levels of polymorphism among the studied parents. Data of these primers recorded a sum of 51 bands. These bands were identified as 29 polymorphic bands and 22 monomorphic ones in all parents under study. The polymorphic bands were scored as 8 unique bands. These unique bands were used to discriminate between the seven squash parents. In addition, the results generated from protein and RAPD profiles were pooled together to elucidate the genetic relationships among the seven examined parents. The constructed dendrogram tree divided the studied parents into two major groups. The first group included Gapla (P_7) only, while the second group was divided into two main sub groups, the first main sub group was divided into two main sub sub groups the first main sub sub group included All Green Bush (P_3) and Courgette Orelia (P_4). The second main sub sub group included Sakiz (P_5) and Copi (P_6). On the other hand, the second main sub group included Eskandrani (P_1) and Zucca Patisson custard white (P_2) parents. From the foregoing results, using protein and RAPD markers for characterization and construction of genetic linkage maps and the molecular genetic diversity of parents support the use of marker-assisted selection (MAS) in squash cultivars breeding programs.
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1.INTRODUCTION

Cucurbitaceae is one of the most important botanical families for human use as favorable vegetable crop. Thus, summer squash (*Cucurbita pepo*, L.) is considered to be one of the most popular vegetable crops grown in Egypt. It is known as a vegetable marrow and is called also Kosa by the Egyptian.

In Egypt, there are only two local cultivars of squash i.e. Balady, which is lately discarded for its prostrate growth habit and low yield and Eskandarani, which is high yielding and satisfies both the producer and consumer.

The past limitation associated with pedigree data and morphological, physiological and cytological markers for assessing genetic diversity in cultivated and wild plant species have largely been circumvented by the development of DNA markers such as random amplified polymorphic DNAs (RAPD).

This method was proved to be useful for germplasm identification, elucidation of genetic relationships of numerous plant cultivars and species, Williams ***et al.*, (1990); Halward *et al.*,(1992) and Levi and Rowland (1997)**. Also, such technique is simple to use and does not require the use of radioactive materials as well as it enables to detect a significant degree of polymorphism. The generated DNA polymorphism reflects both the distance between two annealing sites and the pattern of their distribution throughout the genome of a particular cultivar or species,**Williams *et al.*, (1990)**.

Randomly Amplified Polymorphic DNA analysis (RAPD) could be used to identify many useful polymorphisms quickly and efficiently, and as such, it has a tremendous potential for use in cultivar identification. RAPD analysis has been also used to study genetic relationships in a number of fruit trees as almond ,**Bartolozzi *et al.*, (1998**) plum varieties, **Ortiz *et al.*, (1997**) peach varieties,**Chaparro *et al.*, (1994); Warburton and Bliss(1996**) peach rootstocks ,**Lu *et al.*, (1996)** and **Casas *et al.*, (1999**). RAPD markers have been used in peach genetics and breeding programs

.Chaparro *et al*., (1994); Dirlewanger and Bodo (1994); **Rajapakse *et al*., (1995)**.

Khadari *et al*., (2003) used molecular markers to characterize 100 accessions of olive and to study genetic relationships between them. A total of 497 olive trees were genotyped using 32 RAPD markers. They identified 114 RAPD profiles and detected several cases of mislabeling synonymy and homonymy. This study led to construct a molecular database for the reference collection and to analyze genetic diversity for further prospecting, and for introducing new olive accessions. **Kim and Ko-Kwang (2004)** used RAPD technique to identify 33 Asian pears (*Pyrus* spp.). Nine primers out of 18 primers were produced distinct and reproducible bands. Most of the Asian pears could be identified. The obtained results and Neis genetic distance were used to construct the dendrogram. The Asian pears were differentiated to four clusters. Also, there are quite a few studies concerning the molecular analysis in *Cucurbita* species. The RAPD markers were used to analyze the genetic diversity among *C. moschata* landraces from Korea, southern Africa and other geographical origins, **Baranek *et al.*, (2000).**

Ferriol *et al*., (2003) studied the genetic diversity among 19 Spanish accessions of *C. maxima* using two different molecular marker types: Sequence Related Amplified Polymorphism (SRAP) and RAPD. **Ferriol *et al*., (2004a and 2004b),** in addition to the morphological analysis, employed the SRAP and AFLP (Amplified Fragment Length Polymorphism) molecular markers for analyzing the diversity among a large number of *C. moschata* and *C. maxima* landraces.

Heikal *et al*., (2008) studied two PCR molecular marker techniques; random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) were employed to identify the polymorphisms and the relationships between 14 genotypes, which belong to three different *Cucurbita* species (*C. pepo*, *C. moschata* and *C. maxima*). In RAPD analysis, six random primers revealed a total of 463 fragments, in which 405 (87.5%) were polymorphic. Thirty-one out of 463 RAPD-PCR fragments were found to be useful as genotype specific markers. **Athanasios *et al*., (2009)** studied RAPD markers about winter squash the more reliable clustering of the accessions was accomplished using RAPD markers as well as the combination of the two different data sets, classifying the accessions into three significantly different groups. These groups corresponded to the three different cultivated species of *C. maxima* Duch., *C. moschata* Duch., and *C. pepo* L. Recently, **Dalamu *et al*., (2012)** studied Seventeen RAPD markers about Asian bitter gourd (*Momordica charantia*, L.) to produced 84 amp icons in 50 accessions, of which 33 (41.34 %) were found polymorphic. Joint comparisons among the 50 accessions using Jaccard's similarity coefficient indicated that genetic distances (GD) ranged from 0.03 to 0.28.

The purpose of the present investigation was to study the molecular genetic evaluation of seven squash parents namely: Eskandrani, Zucca Patisson custard white, All Green Bush, Courgette Orelia, Sakiz, Copi and Gapla undre Egyptian condition. The relationship between these parents would be detrmined.

2.MATERIALS AND METHODS

2.1.Materials:

2.1.Samples:

The genetic materials used in the present investigation included seven squash varieties belong to the species (*Cucurbita pepo*, L.). These parental varieties were: Eskandarani (P_1), Zucca Patisson custard white (P_2), All Green Bush (P_3), Courgette Orelia (P_4), Sakiz (P_5), Copi (P_6) and Gapla (P_7). The seeds of these parental varieties were obtained from different countries: (P_1) and (P_6) from Egypt, (P_2) from France, (P_3) from United Kingdom (U.K.), (P_4) from Germany, (P_5) from Turkey and (P_7) from Syria. The seven varieties were chosen to represent a wide rang of variation in most characters.

The characteristics of these parental varieties are summarized as follow: -

Eskandarani (P_1):- A common variety of squash mostly cultivated in Egypt. The plants are standing and intermediate stem length. Fruits are cylindrical, thin and little narrow in the middle. The color is pale or dark green; it is a high yielding variety.

Zucca Patisson custard white (P_2):- The plants are standing, short and have a round flat fruits. The fruit is divided from outside into segments. The fruit color is white.

All Green Bush (P_3): The plants are standing and the fruits color are dark green.

Courgette Orelia (P_4): The plants are standing, long with a yellow color fruits, it is a high yielding variety.

Sakiz (P_5):- The plants are standing, mid early squash variety, has cylindrical fruit shape, light green colored fruit and compact plant provides easy harvest.

Copi (P_6):- A common variety of squash mostly cultivated in Egypt. The plants are standing, the fruit color is pale or dark green, fruit is short and it is a low yielding variety.

Gapla (P_7):- The plants are standing, fruits are light dark color, early production with excellent yield.

In order to insure the purity of these varieties, all these varieties were self pollinated for three successive generations to insure homozygosity and the purity of these varieties at Dokki Experimental Station, Vegetables Breeding Department, Horticulture Research Institute (HRI), Agriculture

Research Center (ARC), Ministry of Agriculture, Egypt.

Differences among fruits of these seven varieties are illustrated in Figure (1).

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Figure 1: Photographic picture represents the differences between the seven parental varieties P_1, P_2, P_3, P_4, P_5, P_6 and P_7 from lift to right.

2.2.Method:-

The analyses were done at Biotechnology Research Lab, Horticulture Research Institute (HRI), Agriculture Research Centre (ARC), Giza, Egypt.

2.2.1.Protein extraction:-

Samples of squash leaves were taken from the seven parental varieties of squash. Total soluble protein were extracted by grounding 0.25g of each sample in 0.9 ml extraction buffer (10ml 0.5MTris pH6.8, 16ml 10% SDS and 30ml D.W.) with shaking thoroughly. The extracts were transferred to Eppendorf tubes and centrifuged for 10 min. at 10000 rpm under cooling. Supernatant were transferred by fresh tubes and used for SDS-PAGE analysis and extraction of isozymes was used as described by **Jonathan and Weeden, (1990).**

2.2.2.Protein related index:-

Fractionation electrophoresis was performed under identical conditions on sodium dedocyl sulphate polyacrylamide gel (SDS-PAGE) (12%W/V) vertical slab using BIORAD Techware 1.5 mm according to the method of **Laemmli (1970)** as modified by **Studier (1973).** The molecular weights of proteins were estimated relative to marker, a wide range molecular weight protein (Fermentas com.).

2.2.3.RAPD-PCR Analysis:-

a. DNA Extraction

Yong and freshly excised tissue were collected separately for each parent. Then DNA extraction was performed as described by **Dellaporta *et al*., (1983)**. About 0.1 gm (fresh weight) of plant tissues was ground to fine powder in liquid N_2 in a mortar. Before the tissue thawed, 1 ml extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA and 0.5 M NaCl) and 0.2 ml 20% SDS were added. The mixture was incubated at 65 °C in water bath for 20 minutes. Then 1 ml of phenol, chloroform and isoamyl alcohol (25: 24: 1) was added. Centrifugation was performed at 10,000 rpm for 10 minutes. The supernatants of each sample were transferred separately to new tubes, and then 1 ml of chloroform and isoamyl (24: 1) was added. Centrifugation was performed at 10,000 rpm for 10 minutes. The supernatants of each sample were transferred separately to a new tube, then 1 ml of isopropanol was added and then kept overnight in a freezer. Centrifugation was performed at 10,000 rpm for 10 minutes. The resulted pellets containing DNA were re-suspended in 1 ml ethanol. Centrifugation was performed at 10,000 rpm for 2 minutes. The DNA pellets were re-suspended in 200 (l TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) buffer. DNA was quantities by quantitatively detemined and gel electrophoresis.

b. Polymerase Chain Reaction (PCR)

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, $MgCl_2$ and Taq polymerase. A total of twenty-one random DNA oligonucleotide primers were independently used according to **Williams *et al*., (1990)** in the PCR reaction. Only five primers succeeded to generate reproducible polymorphic DNA products. **Table 1** lists the base sequences of these DNA primers that produced informative polymorphic bands.

The PCR amplification was performed in a 25 µl reaction volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl_2 (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH_2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Techni TC-512 PCR system. The reaction was subjected to one cycle at 95 ºC for 5 minutes, followed by 35 cycles at 94 ºC for 30 seconds, 37 ºC for 30 seconds, and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 5 minutes. PCR products were run at 100 V for one hour on 1.5 % agarose gels to detect polymorphism between parental varieties of squash under study. After electrophoresis, the RAPD patterns were visualized with UV trans-illuminator. RAPD markers were scored from the gels as DNA fragments present or absent in all lanes. Gels were photographed using a Polaroid camera.

PCR amplification was performed using five random 10 mer arbitrary primers synthesized by (Operon biotechnologies, Inc. Germany) **Table 1**.

2.2.4.Statistical analysis: A randomized complete blocks design was adopted for the present investigation where the data were statistically analyzed using the standard methods according to

Snedecor and Cochran (1980). The new L.S.D. test was used for comparison between means.

The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct dendrogram tree among the studied seven pistachio cultivars. Calculation was achieved using Dice similarity coefficients,**Dice(1945**) as implemented in the computer program SPSS-10.

Table 1: List of the primer names and their nucleotide sequences used in this study

No.	Name	Sequence
1	OP-C04	5´ CCGCATCTAC 3`
2	OP-E19	5´ ACG GCG TAT G 3`
3	OP-G05	5´ CTG AGA CGG A 3`
4	OP-L20	5` TGG TGG ACC A 3`
5	OP-Q15	5` TGC GAT GCG A 3`

3.RESULTS AND DISCUSSION

3.1.Protein Banding Patterns of Leaves:-

The electrophoretic banding pattern of proteins extracted from leaves of the seven parental varieties of squash were shown in **Figure 2** and their densitometric analyses were illustrated in **Table 2** the presence and absence of bands were associated with (1) and (0), respectively.

Results of leaves SDS-PAGE revealed a total number of 18 protein bands with molecular weight (MW) ranging from about 19.0 to 230 KDa. A total of seven common bands (monomorphic bands) were detected, while, the remaining eleven bands were polymorphic with 38.8 % polymorphism.

Protein band with 29 KDa was absent in Gapla (P_7) only which considered as negative specific marker for this parent. There was present band only detected in Eskandrani (P_1) with 27 KDa which considered as positive specific marker for this parent and also, protein band with 21 KDa was absent in Gapla (P_7) only which considered as negative specific marker for this parent.

Table 2: **Densitometric analysis for SDS leaf proteins of seven parents of squash.**

No. of Bands	M.W KDa	Parents						
		1	**2**	**3**	**4**	**5**	**6**	**7**
1	**230**	1	1	0	0	0	0	0
2	**180**	1	1	1	1	0	0	0
3	**85**	1	1	1	1	0	0	0
4	**75**	1	1	1	1	0	0	0
5	**58**	1	1	1	0	0	0	0
6	**55**	1	1	1	1	0	0	0
7	**50**	1	1	1	1	1	1	1
8	**47**	1	1	1	1	1	1	1
9	**40**	1	1	1	1	1	1	1
10	**37**	1	1	1	1	1	1	1
11	**35**	1	1	1	1	1	1	1
12	**33**	1	1	1	1	0	1	0
13	**32**	1	1	1	1	0	1	0
14	**29**	1	1	1	1	1	1	0
15	**27**	1	0	0	0	0	0	0
16	**23**	1	1	1	1	1	1	1
17	**21**	1	1	1	1	1	1	0
18	**19**	1	1	1	1	1	1	1
Total		18	17	16	9	9	11	10

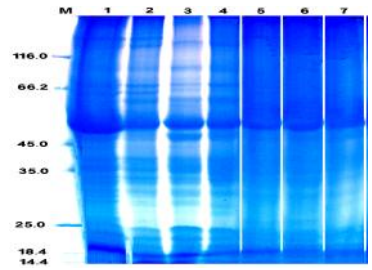

Figure 2: SDS-protein banding patterns of leaf proteins for the seven parents of squa

3.2. Genetic similarity and cluster analysis based on protein markers:-

The protein data were used to estimate the genetic similarity among the seven squash parents by using UPGMA computer analysis **Table 3** The highest similarity index (1.0) was recorded between Eskandrani (P_1) and Zucca Patisson custard white (P_2) the lowest similarity index was detected (0.0) between Gapla (P_7) and both of Eskandrani (P_1) and Zucca Patisson custard white (P_2). A dendrogram for the genetic relationship among the seven parents was carried out as in **Figure (3**) which separated these parents into two major groups. The first group indevided into two main sub groups, the first main sub group included only

Gapla (P_7) and the second one included each of Sakiz (P_5) and Copi (P_6). The second main group devided into two main sub groups, the first main sub group included each of All Green Bush (P_3) and Courgette Orelia (P_4) and the secnod one included Eskandrani (P_1) and Zucca Patisson custard white (P_2).

Table 3: Similarity indices among seven squash parents based on protein analysis.

	1	2	3	4	5	6
1						
2	1.0					
3	0.9	0.9				
4	0.8	0.9	0.9			
5	0.2	0.3	0.3	0.4		
6	0.4	0.5	0.6	0.6	0.8	
7	0.0	0.0	0.1	0.1	0.7	0.5

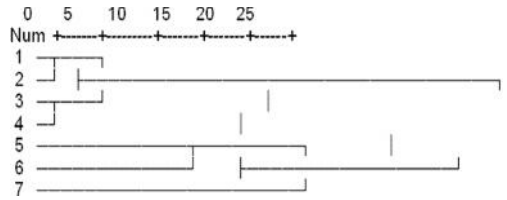

Figure 3: Dendrogram for the genetic distances relationships among seven squash parents based on similarity indices data of protein analysis.

3.3.Molecular genetics identification:-

3.3.1.. Randomly amplified polymorphic DNA (RAPD) markers:-

Data of the amplified fragments using those five 10-mer arbitrary primers for the seven parents succeeded in amplifying DNA fragments **Table 6** and **plate 1**. Polymorphism levels differed from one primer to the other. Primer (OP-E19) exhibited low polymorphism. While, primer OP-L20 showed high polymorphism. On the other hand, primers OP-C04 (41%), OP-G05 (45%) and OP-Q15 (50%) exhibited moderate levels polymorphism which is useful in selected parents of squash identification. The number of total amplified fragments (TAF) and polymorphic bands (PB) for each primer, amplified fragments (AF) and specific markers (SM) for each cultivar using the five primers are shown in **Table (4)**.

There were some specific fragments discriminated each parent from the others as follows:-

Primer OP-L20 showed one specific fragment as negative marker for Courgette Orelia (P_4). On the other hand, Primer OP-Q15 showed one specific fragment as negative marker for Zucca Patisson custard white (P_2).

Table 4: Similarity indices among seven squash parents based on each of protein and RAPD analysis.

	1	2	3	4	5	6
1						
2	.06					
3	.08	0.6				
4	0.5	0.7	1.0			
5	0.4	0.1	0.8	0.7		
6	0.6	0.4	0.7	0.6	0.9	
7	0.0	0.1	0.3	0.2	0.5	0.6

3.3.2.Genetic similarity and cluster analysis based on RAPD markers:-

The RAPD data were used to estimate the genetic similarity among the seven squash parents by using UPGMA computer analysis **Table (4**) .The highest similarity index (1.0) was recorded between Zucca Patisson custard white (P_2) and Sakiz (P_5) and also between Eskandrani (P_1) and Gapla (P_7) while the lowest similarity index was detected (0.0) between All Green Bush (P_3) and Sakiz (P_5) and also between Sakiz (P_5) and Copi (P_6). A dendrogram for the genetic relationship among the seven parents was carried out as in **Figure (5**) which separated into two major groups. The first group included Zucca Patisson custard white (P_2) only, while the second group divided into two main sub groups, the first main sub group included the variety Gapla (P_7) only and the second main sub group included the other parents (P_4), (P_6), (P_5) and (P_3).

The previous results are in harmony with **Kim and Ko-Kwang (2004)** that used RAPD technique to identify 33 Asian pears (*Pyrus* spp.). Nine primers out of 18 primers produced distinct and reproducible bands. Most of the Asian pears could be identified. The obtained results and Neis genetic distance were used to construct the dendrogram. The Asian pears were differentiated to four clusters. Also, results are in line with **Goto *et al.*, (1998) ; Loureiro *et al.*, (1998)** who showed that grape vine cultivars were easily discriminated usingAFLP, RFLP and RAPD techniques.

Table 5: Similarity indices among seven squash parents based on RAPD analysis

	1	2	3	4	5	6
1						
2	0.7					
3	0.7	0.7				
4	0.3	.05	0.1			
5	0.4	1.0	0.0	0.1		
6	0.3	0.7	0.2	0.3	0.0	
7	1.0	0.9	0.6	0.7	0.7	0.4

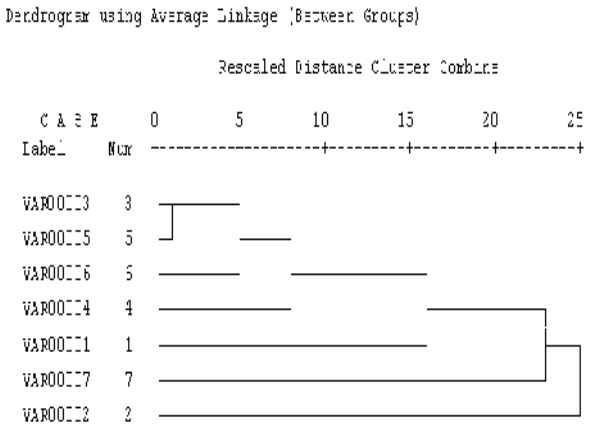

Figure 4: Dendrogram for the genetic distances relationships among seven squash parents based on similarity indices data of RAPD analysis.

3.3.3. Combined identification based on protein and RAPD analysis:-

Genetic similarities and phylogenetic relationships between the seven parental squash varieties based on each of protein and RAPD markers **Table (5)** were determined by using UPGMA computer program.

The highest similarity index recorded was (1.0), between All Green Bush (P_3) and Courgette Orelia (P_4), while the lowest similarity index recorded was (0.0), between Eskandrani (P_1) and Gapla (P_7) parental varieties.

The phylogenetic dendrogram based on the protein and RAPD markers **Figure 5** separated the seven parents of squash into two major groups. The first group included only Gapla (P_7), while the second group was divided into two main sub groups, the first main sub group was divided into two main sub sub groups the first one included All Green Bush (P_3) and Courgette Orelia (P_4) and the second one included Sakiz (P_5) and Copi (P_6). On the other hand, the second main sub group included Eskandrani (P_1) and Zucca Patisson custard white (P_2) parental varieties. Also, combined analysis results are in line with **Dalamu *et al.*, (2012)** who showed that the combined data analysis of RAPD and ISSR markers indicated that the relative polymorphism among accessions was 52.6 % with 2.64 polymorphic amplicons per primer. The value of average polymorphic information content, resolving power and marker index were 0.26, 1.42, and 1.33, respectively. These data demonstrate a large genetic variability among the Asian bitter gourd genotypes examined, which indicates that they should be considered as a valuable gene pool for bitter gourd breeding programs.

Table 6: Amplified bands, polymorphic bands, monomorphic bands and polymorphic percentage of protein and RAPD analysis for seven parents of squash

Primer Name	Amplified Bands	Polymorphic Bands	Monomorphic Bands	Unique Bands	Polymorphic %
OP-C04	12	5	7	-	41.6
OP-E19	6	2	4	-	66.6
OP-G05	10	5	5	1-	50.0
OP-L20	9	8	1	3-	88.8
OP-Q15	14	9	5	4-	64.2
Protein	18	11	7	+1 , -2	61.0
Total	69	40	29	11	57.9

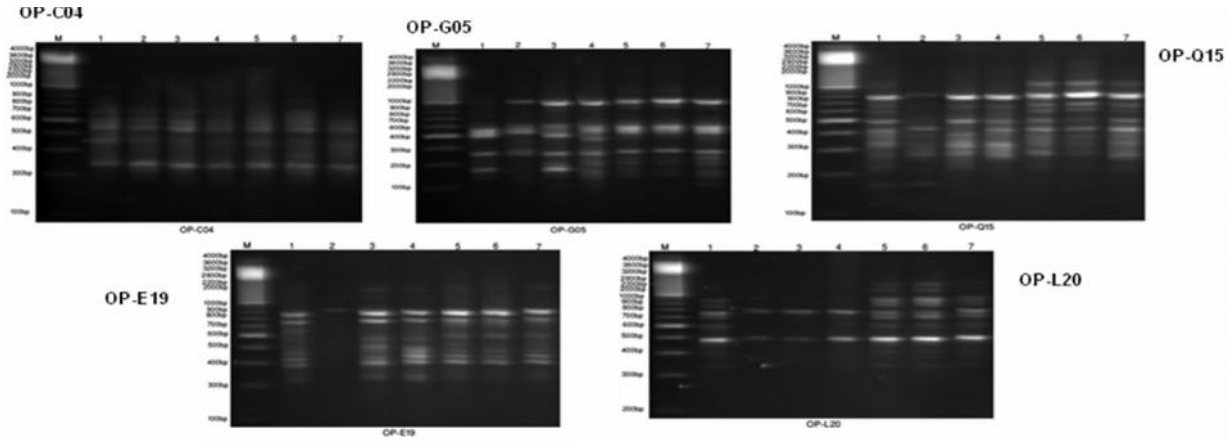

Plate 1: RAPD profiles of seven squash parents amplified with five primers for each analysis

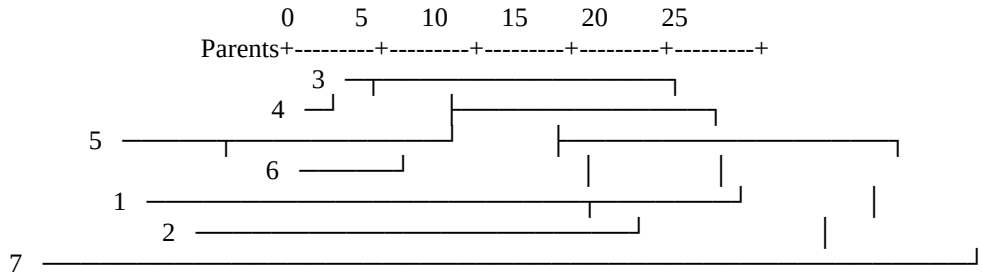

Figure 5: Dendrogram for the genetic distances relationships among seven squash parents based on similarity indices data of protein and RAPD analysis

4. REFERENCES

1. **Athanasios, L.T.; O. Koutita; A. Anastasiadou; G.N. Skaracis; E.T. Mavrona and M.K. Sotiriou (2009).** Description and analysis of genetic diversity among squash accessions. Braz. Arch. Biol. Technol. V.52 n.2: pp. 271-283, Mar/Apr 2009.
2. **Baranek, M.; G. Stift; J. Vollmann and T. Lelley (2000).** Genetic diversity within and between the species *Cucurbita pepo*, *C. moschata* and *C. maxima* as revealed by RAPD markers. *Cucurbit. Genet. Coop. Rep.*, 23, 73-77.
3. **Bartolozzi, F.; M.L. Warburton; S. Arulsekar and T.M. Gradziel (1998).** Genetic characterization and relatedness among California almond cultivars and breeding lines detected by Randomly Amplified Polymorphic DNA (RAPD) analysis. J. Amer. Soc. Hort. Sci., 123(3): 381-387.
4. **Casas, A.M.; E. Igartua; G. Balaguer and M.A. Moreno (1999).** Genetic diversity of Prunus root stocks analyzed by RAPD markers. Euphytica. 110: 139-149.
5. **Chaparro, J.X.; D.J. Werner; D.O. Malley and R.R. Sederoff (1994).** Targeted mapping and linkage analysis of morphological, isozyme and RAPD markers in peach. Theor. Appl. Genet., 85: 805-815.
6. **Dalamu, T.K.B.; A.B. Gaikwad; S. Saxena; C. Bharadwaj and A.D. Munshi (2012).** Morphological and molecular analyses define the genetic diversity of Asian bitter gourd (*Momordica charantia*, L.). AJCS 6(2):261-267.
7. **Dellaporta, S.L.; J. Wood and J.B. Hicks (1983).** A plant DNA mini preparation. Version III. Plant Mol. Biol., Rep.1: 19-21.
8. **Dice, L.R. (1945).** Measures of the amount of ecologic association between species. Ecology, 26: 297-302.
9. **Dirlewanger, E. and C. Bodo (1994).** Molecular genetic mapping of peach. Euphytica 77: 101-103.
10. **Ferriol, M.; B. Picó and F. Nuez (2003).** Genetic diversity of some accessions of *Cucurbita maxima* from Spain using RAPD and SBAP markers. Genet. Resour. Crop E. Vol. 50: 227-238.
11. **Ferriol, M.; B. Picó and F. Nuez (2004a).** Morphological and molecular diversity of a collection of *Cucurbita maxima* landraces. J. Amer. Soc. Hort. Sci., 129 (1), 60-69.
12. **Ferriol, M.; B. Picó; P. Fernández and F. Nuez (2004b).** Molecular diversity of a germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. *Crop Sci.*, 44, 653-664.
13. **Goto, Y.M.; R. Ochioka; L. Bonian; K. Hashizume; Y. Umda and S. Horiuchi (1998).** RFLP and RAPD analysis of caviled cultivated grapes. J. Jap. Soc. Hort., 67:483-490.
14. **Halward, T.; T. Stalker; E. LaRue and G. Kochert (1992).** Use of single primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea*, L.). Plant Mol. Biol. 18: 315-325.
15. **Heikal, A. Hadia; H.S. Abdel-Razzak and E.E. Hafez (2008).** Assessment of Genetic Relationships among and within *cucurbita* Species Using RAPD and ISSR markers. J. Appl. Sci. Res., 4(5): 515-525.
16. **Jonathan, F.W. and N.F. Weeden (1990).** Visualization and interpretation of plant isozymes. Isozymes in Plant Biology. D.E. Soltis and P.S. Soltis (eds.). London Chapman and Hall, pp. 5-45.
17. **Khadari, B.; C. Breton; N. Moutier; J.P. Roger; G. Besnard; A. Berville and F. Dosba (2003).** The use of molecular markers for germplasm management in a French olive collection. Theor. Appl. Genet. 106: 521-529.
18. **Kim, D. and C. Ko-Kwang (2004).** Identification markers and phylogenetic analysis using RAPD in Asian pears (*Pyrus* spp.). J. Korean Soc. Hort. Sci. 45 (4): 194-200.
19. **Laemmli, U.K. (1970).** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
20. **Levi, A. and L.J. Rowland (1997).** Identifying blueberry cultivars and evaluating their genetic relationships using randomly amplified polymorphic DNA (RAPD) and

simple sequence repeat (SSR) anchored primers. J. Amer. Soc. Sci. 122 (1): 74-78.

21. **Loureiro, M.D.; M.C. Martinez; J.M. Boursiquot and P. This (1998).** Molecular marker analysis of *Vitis vinifera* and some similar grapevine cultivars. J. Amer. Soc. Hort. Sci., 123(5):842-848.
22. **Lu, Z.; G.L. Reighard; W.V. Baird; A.G. Abbott; S. Rajapakse and Z.X. Lu (1996).** Identification of peach rootstock cultivars by RAPD markers. J. Hort. Sci. 31 (1): 127-129.
23. **Ortiz, A.; R. Renaud; I. Calzada and E. Ritter (1997).** Analysis of plum cultivars with RAPD markers. J. Hort. Sci. 72 (1): 1-9.
24. **Rajapakse, S.; L.E. Belthoff; G. He; A.E. Estager; R. Scorza; I. Verde; R.E. Bellard; W.V. Baird; R. Callahan; A. Monet and A.G. Abbott (1995).** Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor. Appl. Genet., 90: 503-510.

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25. **Snedecor, G.W. and W.G. Cochran (1980).** Statistical Methods 7th Ed. The Iowa State Univ. Press Ames. Iowa, USA.
26. **Studier, F.W. (1973).** Analysis of Bacteriophage T4 early RNAs and proteins of slab gel. J. Mol. Bio. 79:237-248.
27. **Warburton, M.L. and F.A. Bliss (1996).** Genetic diversity in peach (*Prunus persica*, L. Batch) revealed by randomly amplified polymorphic DNA (RAPD) markers and compared to inbreeding coefficients. J. Amer. Soc. Hort. Sci. 121 (6): 1012-1019.
28. **Williams, J.G.K.; A.R. Kubelk; K.J. Livak; J.A. Rafalski and S.V. Tingey (1990).** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acid Res, 18: 6231-6235.