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Screening some Broccoli and Cabbage Genotypes Biodiversity Using Randomly Amplified Polymorphic DNA (RAPD).

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Abstract:

Ten RAPD markers were used to detect the genetic variability and relationships among four broccoli and three cabbage genotypes. The results of RAPD analysis showed that all the 5 primers surveyed detected polymorphism for all broccoli genotypes. A total of 39 DNA bands were amplified by the 5 primers from all genotype and 21 of these fragments showed polymorphism (53.85%). The rest of these bands (46.15%) were common between the four genotypes . On the other hand, all of the 7 primers surveyed, used with cabbage, detected polymorphism among all cabbage genotype. A total of 69 DNA bands were amplified by the 7 primers from all genotypes and 23 of these fragments showed polymorphism (33.33%). The rest of these bands (66.67%) were common between the three genotypes. The investigation suggested that the RAPD approach showed considerable potential for identifying and discriminating broccoli and cabbage genotypes.

Key words: *Brassica oleracea* , genetic markers ,varietal identification, DNA polymorphis

Introduction:

Cabbage (*Brassica oleracea var. capitata).* (2n = 2x = 18) belongs to the Brassicaceae family and is one of the most important vegetables in the world due to its wide adaptation, high yield, long shelf time, and high economic significance. **(Liwang Liu1 *et al*, 2007)**. In Egypt most of the people know the cabbage nutrient value but don't know about the nutrient value of broccoli

Broccoli *Brassica oleracea* var. *italica* is a minor vegetable crop cultivated in a very small area over all Egypt. No statistics were found to determine such area in Egypt not even in F.A.O. statistics data base. Broccoli is highly nutritious, and has been deemed as a vegetable with potential anti-cancer activity due to high levels of glucoraphanin, which can hydrolyses to form sulphoraphane, an isothiocyanate. Broccoli sprouts have been reported to have 20–50 times the glucoraphanin concentration of mature broccoli heads **(Fahey *et al*., 1997).** Moreover, dietary antio xidants, vitamins and

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non-nutrient components such as flavonoids, are present in crucifers and may decrease the risk for certain cancers **(Lindsay and Astley, 2002)**.

The randomly amplified polymorphic DNA (RAPD) markers generated by polymerase chain reaction (PCR) is technically the simplest, less expensive, fast and does not require prior knowledge of the target sequences for the design of primers (**Williams *et al.*, 1990**). The RAPD markers have already been used in *Brassica oleracea* for the assessment of genetic variability, diversity and fingerprinting of broccoli and cabbage genotypes (**Jinguo and Carlos , 1991; Janel *et al.* (2002) and Qin *et al.* (2007).**

The objective of this investigation was to study the performance of four broccoli and three cabbage genotype plants under Assiut conditions and to determine the genetic differences between the four broccoli and the three cabbage genotypes using ten RAPD markers.

Materials and methods:

II-Random amplified polymorphic DNA (RAPD) analysis:

2.1- DNA extraction:

Field experiments were conducted at the Experimental Farm of Faculty of Agriculture, Assiut, Egypt in two consecutive winter seasons of 2008/2009 and 2009/2010 to grow different genotypes of broccoli and cabbage. The isolation of total cellular DNA was performed on the basis of a modified CTAB protocol for plants containing high polyphenol components designed by **Porebski *et al.*, (1997)**. Four broccoli and three cabbage genotypes (Table1) plants fresh leaves were collected, frozen in liquid nitrogen, and stored at -70ºC until use. Sample tissues (0.5g) from each donor plant were grinded using mortar and pestle in the presence of liquid nitrogen until finally ground. Frozen ground leaf tissues were transferred to 15 ml polyptopylene centrifuge tubes. Then, 5.0 ml of 60ºC extraction buffer (100 mM tris, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 2% CTAB (hexadecyltrimethylammonium bromide), 0.3% β-mercaptoethanol) and 50 mg Polyvinylpolyrrolidone (PVP) were added to the samples. Then mixed by inversion and incubated in 60ºC oven (with shaking) for 25 to 60 minutes.

The samples were removed from heat and cooled to room temperature for 4-6 min. A mixture (6.0 ml) of chloroform: octanol (24:1) were then added to the samples and mixed by inversion to form emulsion. The samples were centrifuged at 3000 rpm for 20 min. at room temperature. An aqueous solution was transferred to new 15 ml centrifuge tubes using wide-bore pipette. The step of chloroform: octanol was repeated to remove cloudiness (PVP) in aqueous phase. A half volume of 5 M NaCl were added to the final

aqueous solution recovered. In addition, two volumes of cold (-20ºC) 95% ethanol were added to solution. Samples were placed in freezer (-20ºC) for 10 min. or left at 4-6ºC overnight to accentuate precipitation. After then, samples were centrifuged at 3000 rpm for 6 min.

The supernatant was poured and pellet washed with cold (0-4ºC) 79% v/v ethanol, then pellet dried in 37ºC oven and dissolved in 300µl TE buffer (10 mM tris-HCl, 1 mM EDTA, pH 8.4) overnight at 4-6ºC. Dissolved pellet was transferred to 1.5 ml Eppendorf tubes and 3µl RNase A (10 mg/ml) was added and incubated in 37ºC water bath for approximately 1 hour. The samples were treated with 3µl proteinase-K (1mg/ml), and incubated at 37ºC for 15 to 30 minutes. Equal volumes of phenol and chloroform (150µl each) were added to each Eppendorf tube, vortexed briefly and spinned (in microcentrifuge) at 14,000 rpm for 10 to 15 minutes. Upper layers were collected in new 1.5 ml Eppendorf tube and then 50 µl TE were added to phenol phase. The last step was repeated.

Sodium acetate (1/10 vol. of 2M) and absolute ethanol (2 vol.) were added, mixed and left overnight in freezer (-80ºC). Then samples were centrifuged at 14,000 rpm for 10 to 20 minutes, drained and washed with70% v/v ethanol. Ethanol was removed, tubes were dried and 100 to 200 µl TE buffer were added. DNA concentrations were measured using a Hoefer Quanta 200 Fluorometer and 0.5 ng DNA dilutions were prepared for RAPD-PCR analysis.

2.2- <u>Quantitation of DNA using spectrophotometry</u>:

DNA concentration was determined using the spectrophotometer. DNA concentration of most solutions was measured by using the conversion factor that 1.0 OD at 260 nm is equivalent to 50 µg/ml DNA. Pure preparations of DNA have an OD_{260}/OD_{280} value of 1.8, if the samples are contaminated with protein or phenol, the OD_{260}/OD_{280} will be significantly less than the 1.8 value. So, an appropriate measure of the purity of the isolated DNA was determined using the $A_{260}/_{280}$ ratio.

2.3- <u>RAPD markers assay</u>:

RAPD marker assays are based on the PCR amplification of random locations in the plant genome. The DNA amplification protocol was performed as described by **Williams *et al.*, (1990)** with some modifications.

2.3.1- <u>Primers and DNA marker used in RAPD analysis</u>:

Oligonucleotide sequences of ten, 10-mer, random primers used in this study were selected from a set of Operon kits (A,B, and E) were used for broccoli and (A, B, D, E and O) were used for cabbage (Operon Technologies Inc., Alameda CA) the codes and sequences of these primers are shown in table (2 and 3). A100 bp Ladder (Amersham Pharmacia) was used in RAPD analysis, this marker covers a

range of DNA fragment size between 3000 bp to 250 bp.

2.3.2- <u>Preparation of PCR reactions:</u>

Reactions were carried out in a 25 µl volume of 3 µl containing 10 ng/ µl of genomic DNA template, 3 µl moles of each primer, 2.5 µl 10x buffer 2.5 µl each of dNTPs (2mM), 2 µl $MgCl_2$ (25 mM), 0.001 gelatin and 0.3 0f 5 units /µl Taq polymerase (Appligene). A master mix was prepared in a 1.5ml microfuge tube, according to the number of PCR reactions to be performed, with an extra reaction include compensating for the loss of part of the solution due to frequent pipetting. An aliquot of 47.5 µl master mix solution was dispensed in each PCR tube (0.2ml), containing 2.5µl of the appropriate template DNA, so that each reaction contained:

Component	Amount of one PCR reaction
H_2O	11.7µl
10X reaction buffer	2.5µl
dNTP's mix	2.5µl
$MgCl_2$	2.0µl
Primer	3.0µl
Taq polymerase	0.3µl
Template DNA	3.0µl
Total volume	**25.0µl**

Table (1): The name and sources of the four broccoli and the three cabbage genotypes used in this study.

	Genotypes	Source
Broccoli:		
1-	Assiut 1	Aboul-Nasr *et al.* (2008)
2-	Calabrese U.S.A.	West Hills,U.S.A
3-	Calabrese France.	Bourget et Sanvoisin, France.
4-	Italian.	Battistini Sementi s.n.c. Italy.
Cabbage:		
1-	Balady Mohassan	Mecca, Trade
2-	Balady	Harraz Company, Cairo, Egypt
3-	Brunswick	GSN Semences France

Table (2): Sequence and primers codes of the random primers used to study variation in five broccoli donors.

No.	Primer codes	Sequence (5' to 3')
1	OPB-10	CTGCTGGGAC
2	OPA-09	GGGTAACGCC
3	OPB-08	GTCCACACGG
4	OPA-16	AGCCAGCGAA
5	OPE-04	GTGACATGCC

Table (3): Sequence and primers codes of the random primers used to study variation in five cabbage donors.

No.	Primer codes	Sequence (5' to 3')
1	OPB-10	CTGCTGGGAC
2	OPB-01	GTTTCGCTCC
3	OPA-17	GACCGCTTGT
4	OPE-04	GTGACATGCC
5	OPE-05	TCAGGGAGGT
6	OPD-05	TGAGCGGACA
7	OPO-14	AGCATGGCTC

2.3.3- PCR program and temperature profile:

The PCR temperature profile was applied through a Gene Amp® PCR System 9700 (Perkin Elmer, England). Amplification of DNA was carried out in a thermocycler programmed for 40 cycles as follows: 94ºC for 5 min (1 cycle), 94ºC for 40 sec. 36ºC for 1 min, 72ºC for 1 min, (40 cycles), 72ºC for 7 min (last cycle) then followed by soaking at 4ºC.

2.4-Gel electrophoresis:

The products obtained in reaction (25µl) and 100bp Ladder marker were separated on the 1.5% agarose containing 0.5 µg/ml of ethidium bromide in 1x concentrated TBE buffer (89 mM Tris-borate; 2.5 mM EDTA) at 95 voltage for approximately 2.5 hours. The patterns were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000) in order to document the results for further analysis.

2.5- Data analysis:

Agarose gel photos were scanned by Gene Profiler 4.03 computer software program that uses automatic lane and peak

finding to detect the presence of bands in a gel, and calibrate them for size and intensity. A binary data matrix containing the presence (1) or the absence (0) of bands was made. The software package **MVSP** (**M**ulti-**V**ariate **S**tatistical **P**ackage) was used and genetic similarities computed using the Dice coefficient of similarity as in Nei & Li (**1979**):

$$\text{Similarity} = \frac{2 \text{ X } n11}{(2 \text{ X } n11) + n01 + n10}$$

Where: n11 - designates the number of common bands for two compared samples,

n10- cases where the bands were visible only in the first sample,

n01 – when they were visible in the other sample only.

The genetic distance between donor parents and within each parent and its regenerated plantlets were estimated using natural logarithm (-ln F) of the similarity estimates. Cluster analysis was carried out on similarity estimates using the unweighted pair-group method with arithmetic average (UPGMA). These methods were carried out through MVSP software programs. The results were then represented as dendrogram for each primer and all primers.

Results and Disscusion

Random amplified polymorphic DNA (RAPD) for broccoli

Randomly amplified polymorphic DNA (RAPD) technique requires only the presence of single "randomly chosen" oligonucleotides. The ability of RAPDs to produce multiple bands using a single primer means that a relatively small number of primers can be used to generate a very large number of fragments. These fragments are usually generated from different regions of the genome and hence multiple loci may be examined very quickly **(Williams *et al.*, 1990; Martin *et al.*, 1991; Edwards 1998; Piola *et al.*, 1999 and Ovesna *et al.*, 2002).**

In the present investigation, 5 random 10-mer primers (OPB-10, OPA-09, OPB-08, OPA-16 and OPE-04) were used to study the genetic differences and relationships among the four genotypes of broccoli (Table 2). The 5 primers amplified a total of 39 DNA fragments from all tested genotypes and ranged in size from 2406 bp(OPE-04) to 244 bp (OPB-10) (Table 4 and Fig.1).

The highest number of amplified DNA fragments was detected for the Primer OPA-09 (10 bands), while the lowest number was amplified with the primer OPB-08 (5 bands), in (table 5).

Calabrese France genotype displayed the lowest number of DNA fragments (27 bands), while the Italian genotype revealed the highest number of bands (33 bands). These variation in the number of bands amplified by different primers influenced by variable factors such as primer structure and number of annealing sites in the genome **(Kernodle *et al.*, 1993)**, in (table 5).

All the 5 primers surveyed detected polymorphism for all broccoli genotypes. A total of 39 DNA bands were amplified by

the 5 primers from all genotype and 21 of these fragments showed polymorphism (53.85%). The rest of these bands (46.15%) were common between the five lines. The monomorphic bands are constant bands and cannot be used to study the diversity while polymorphic bands revealed differences and could be used to examine and establish systematic relationships among the genotypes **(Hadrys *et al.*, 1992).**

Unique DNA fragments with different sizes were detected in particular lines but not in the others using different primers. The presence of a unique band for a given line is referred as positive marker while the absence of a common band served as negative marker. Such bands could be used as DNA markers for line identification and discrimination.

In this respect, one DNA fragments in genotype Calabrese U.S.A [807 bp (OPA-16)], two band in genotype Calabrese France [552bp (OPB-08) and 1070bp (OPA-16)] and one band in the Italian genotype [726bp (OPA-16)] were line-specific positive markers (Table (6). Line-specific negative markers were also recorded for genotype Assiut1 [1690bp (OPA-16)] and five bands for genotype Calabrese France [268bp (OPA-09), 488bp (OPB-08) and 1525bp, 930bp and 830bp (OPE-04)]. The higher number of RAPD population-specific markers was generated by the primer OPA-16 (3 markers), followed by OPB-08 (1 marker). (Table 6).

Clustering values of the four broccoli genotypes resulting from the UPGMA are given in Table (7). The analysis was based on the number of markers that were different between any given pair genotypes. The dendrogram showed that both Calabrese U.S.A. and Italian. were clustered together firstly with 0.79 genetic similarity while, the second cluster included Assiut1 and Calabrese France at 0.64 similarity. These results suggested that the RAPD approach showed considerable potential for identifying and discriminating the four broccoli genotypes.

Table (4): Survey of the RAPD-DNA fragments of the seven primers in four broccoli genotypes.

No	Primers	bp	Assiut1	Calabrese U.S.A.	Calabrese France.	Italian.
1		1772	-	-	+	+
2		1080	+	+	+	+
3		994	+	+	+	+
4		744	+	+	+	+
5	OPB-10	581	+	+	+	+
6		473	+	+	+	+
7		435	+	+	+	+
8		244	+	+	+	+
9		1778	+	+	+	+
10		1579	+	+	+	+
11		1247	-	-	+	+
12		1024	+	+	+	+
13		664	+	+	+	+
14	OPA-09	590	+	+	+	+
15		484	+	+	-	-
16		398	+	+	+	+
17		314	-	-	+	+
18		268	+	+	-	+
19		1846	-	-	+	+
20		1235	+	+	+	+
21	OPB-08	879	+	+	+	+
22		552	-	-	+	-
23		488	+	+	-	+
24		1690	-	+	+	+
25		1148	-	+	-	+
26		1070	-	-	+	-
27		929	-	+	-	+
28	OPA-16	807	-	+	-	-
29		726	-	-	-	+
30		631	+	+	+	+
31		511	+	+	+	+
32		2406	+	-	-	+
33		1525	+	+	-	+
34		930	+	+	-	+
35	OPE-04	830	+	+	-	+
36		660	+	+	+	+
37		546	+	-	-	+
38		359	+	-	+	-
39		275	+	-	+	-
Total DNA Fragments			28	28	27	33

Table (5): Number of amplified DNA-fragments and polymorphic bands in four broccoli genotypes investigated with five RAPD primers.

Primers Code	No. of amplified bands				Total amplified bands	No. of polymorphic bands	% of polymorphic bands
	Assiut1	Calabrese U.S.A.	Calabrese France.	Italian.			
OPB-10	7	7	8	8	8	1	12.5
OPA-09	8	8	8	9	10	4	40.0
OPB-08	3	3	4	4	5	3	60.0
OPA-16	2	6	4	6	8	6	75.0
OPE-04	8	4	3	6	8	7	87.5
TOTAL	28	28	27	33	39	21	53.85

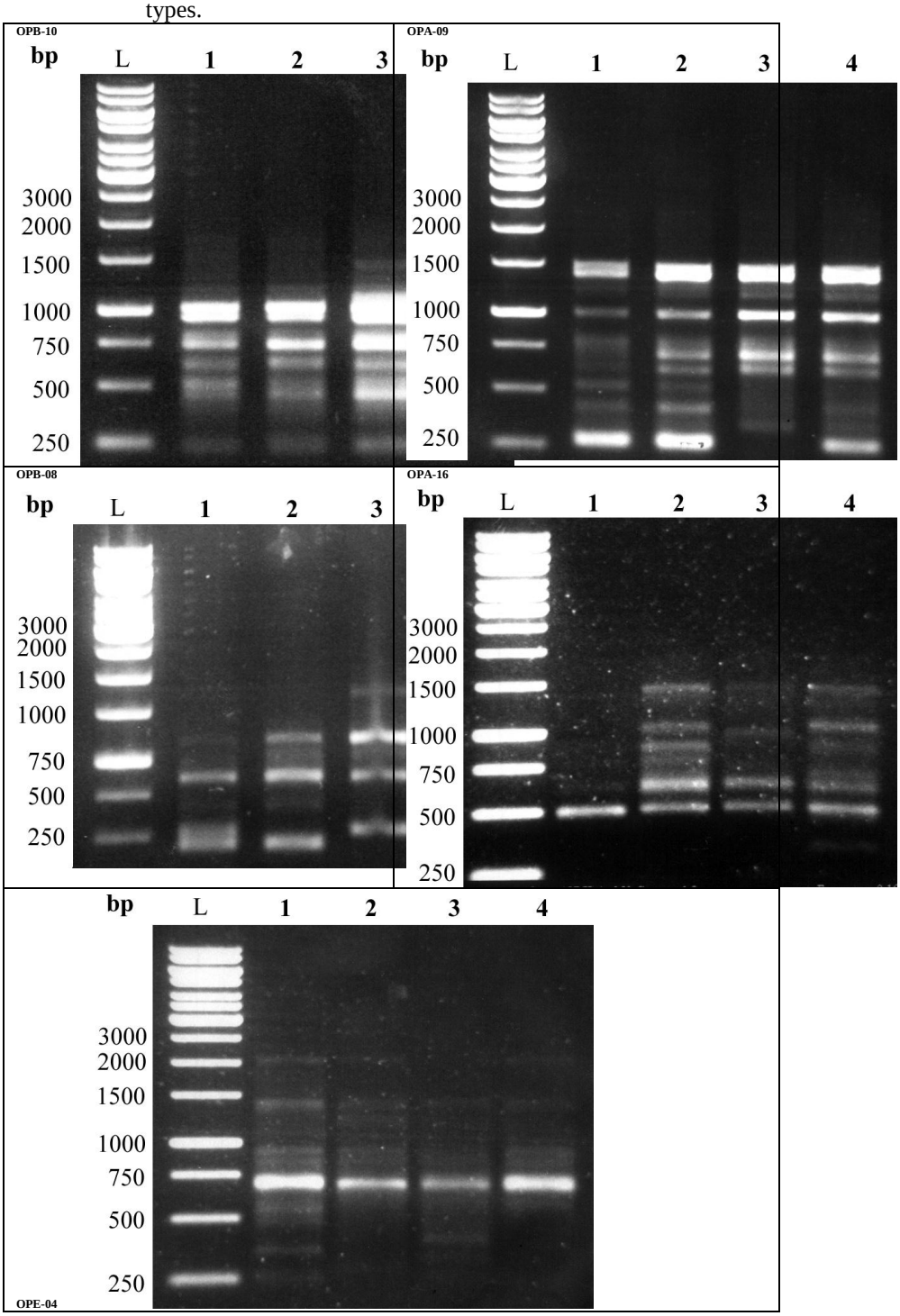
Table (6): Four broccoli genotypes characterized by unique positive and/or negative RAPD markers, marker size and total number of markers.

Primer	As-siut1		Cala-brese U.S.A.		Cala-brese Franc e.		Ital-ian.		Positive marker	Negative marker	Total markers
	Positive marker	Nega-tive marker	Positive marker	Nega-tive marker	Positive marker	Nega-tive marker	Positive marker	Nega-tive marker			
OPB-10											
OPA-09						268				1	1
OPB-08					552	488			1	1	2
OPA-16		1690	807		1070		726		3	1	4
OPE-04						1525 930 830				3	3
Total		1	1		2	5	1		4	6	
	1		1		7		1				10

Table (7): Genetic similarity values of four broccoli genotypes calculated from 39 DNA fragments generated with five primers

Similarity matrix	Assiut1	Calabrese France.	Italian.	Calabrese U.S.A.
1	1			
3	0.636	1		
4	0.310	0.409	1	
2	0.286	0.286	0.794	1

Fig. (1): Agarose gel electrophoresis of RAPD profile in four broccoli genotypes.



Random amplified polymorphic DNA (RAPD) for cabbage

In the present investigation, 7 random 10-mer primers (OPE-04, OPE-05, OPO-14, OPA-17, OPB-10, OPD-5, OPB-01) were used to study the genetic differences and relationships among the three genotypes of Cabbage (Table 3). The 7 primers amplified a total of 69 DNA fragments from all tested lines and ranged in size from 3915 bp (OPA-17) to 360 bp (OPE-05) (Table 8 and Fig.2).

The highest number of amplified DNA fragments was detected for the Primer OPB10, OPD-05 and OPE-05 (13 bands each), while the lowest number was amplified with the primer OPA-17 (4 bands), in (Table 9).

Brunswick genotype displayed the lowest number of DNA fragments (58 bands), while the Balady Mohassan and Balady genotypes revealed the highest number of bands (60 bands each). These variation in the number of bands amplified by different primers influenced by variable factors such as primer structure and number of annealing sites in the genome **(Kernodle *et al.*, 1993)**.

All of the 7 primers surveyed detected polymorphism among all cabbage genotype. A total of 69 DNA bands were amplified by the 7 primers from all genotypes and 23 of these fragments showed polymorphism (33.33%). The rest of these bands (66.67%) were common between the three genotypes. The monomorphic bands are constant bands and cannot be used to study the diversity while polymorphic bands revealed differences and could be used to examine and establish systematic relationships among the genotypes **(Hadrys *et al.*, 1992).**

Unique DNA fragments with different sizes were detected in particular lines but not in the others using different primers. The presence of a unique band for a given line is referred as positive marker while the absence of a common band served as negative marker. Such bands could be used as DNA markers for line identification and discrimination.

In this respect, four DNA fragments in genotype 1 [2052bp (OPB-01)], 2346bp (OPB-10), 1580bp (OPE-04) and 457bp (OPE-05), two band in genotype 2 [2803bp, 471bp (OPD-05) and six bands for genotype 3 [1570bp (OPB-01), 1642bp and 1051bp (OPD-05), 608bp (OPE-05) and 2007bp, 1028bp (OPO-14)] were line-specific positive markers (Table (10). Line-specific negative markers were also recorded for genotype 1 [1257pb (OPB-01) and 736bp (OPE-04)], one band for 2 [580bp (OPO-14) and seven bands for genotype 3 [1642bp, 1005bp (OPB-01), 1642bp (OPB-10), 3915bp (OPA-17), 1099bp (OPD-05), 553bp (OPE-05) and 2672bp (OPO-14). The higher number of RAPD population-specific markers were generated by primers OPB01 OPD05 (5 markers each),

followed by OPO14 (4 marker). (Table 10)The lowest number of RAPD population-specific markers was generated by primer OPA17 (one marker).

Clustering values of the three Cabbage genotypes resulting from the UPGMA are given in (Table 11). The analysis was based on the number of markers that were different between any given pair lines. The dendrogram showed that both Balady Mohassan and Balady were clustered together firstly with 0.92 genetic similarity .These results suggested that the RAPD approach showed considerable potential for identifying and discriminating the three cabage broccoli genotypes.

Table (8): Survey of the RAPD-DNA fragments of the seven primers in three cabbage genotypes.

No	Primers	bP	B.M.	B	Br.
1	POE-04	2672	+	+	+
2		2316	+	+	+
3		2105	+	+	+
4		1824	+	+	+
5		1580	+	-	-
6		1370	+	+	+
7		1245	+	+	+
8		980	+	+	+
9		850	+	+	+
10		736	-	+	+
11	POE-05	3084	+	+	+
12		2105	+	+	+
13		1913	+	+	+
14		1739	+	+	+
15		1437	+	+	+
16		1306	+	+	+
17		1028	+	+	+
18		850	+	+	+
19		702	+	+	+
20		608	-	-	+
21		553	+	+	-
22		457	+	-	-
23		360	+	+	+
24	POO-14	2672	+	+	-
25		2316	+	+	+
26		2007	-	-	+
27		1824	+	+	+
28		1580	+	+	+
29		1187	+	+	+
30		1028	-	-	+
31		891	+	+	+
32		772	+	+	+
33		580	+	-	+

34	POA-17	3915	+	+	-
35		2803	+	+	+
36		2316	+	+	+
37		1913	+	+	+
38	POB-10	2346	+	-	-
39		1502	+	+	+
40		1642	+	+	-
41		1795	+	+	+
42		1257	+	+	+
43		1099	+	+	+
44		962	+	+	+
45		880	+	+	+
46		769	+	+	+
47		704	+	+	+
48		589	+	+	+
49		493	+	+	+
50		377	+	+	+
51	POD-05	2803	-	+	-
52		2346	+	+	+
53		1502	+	+	+
54		1642	-	-	+
55		1877	+	+	+
56		1374	+	+	+
57		1202	+	+	+
58		1099	+	+	-
59		1051	-	-	+
60		841	+	+	+
61		673	+	+	+
62		471	-	+	-
63		361	+	+	-
64	POB-01	2803	+	+	+
65		2052	+	-	-
66		1642	+	+	-
67		1570	-	-	+
68		1257	-	+	+
69		1005	+	+	-

B.M. = Balady Mohassan
B. = Balady
Br. = Brunswick

Table (9): Number of amplified DNA-fragments and polymorphic bands in three Cabbage genotypes investigated with seven RAPD primers.

Primers Code	No. of amplified bands			Total amplified bands	No. of polymorphic bands	% of polymorphic bands
	B.M.	B.	Br.			
OPE-04	9	9	9	10	2	20.00
OPE-05	12	11	11	13	3	23.08
OPO-14	8	7	9	10	4	40.00
OPA-17	4	4	3	4	1	25.00
OPB-10	13	12	11	13	2	15.83
OPD-05	9	11	9	13	6	46.15
OPB-01	4	4	3	6	5	83.33
TOTAL	60	60	58	69	23	33.33

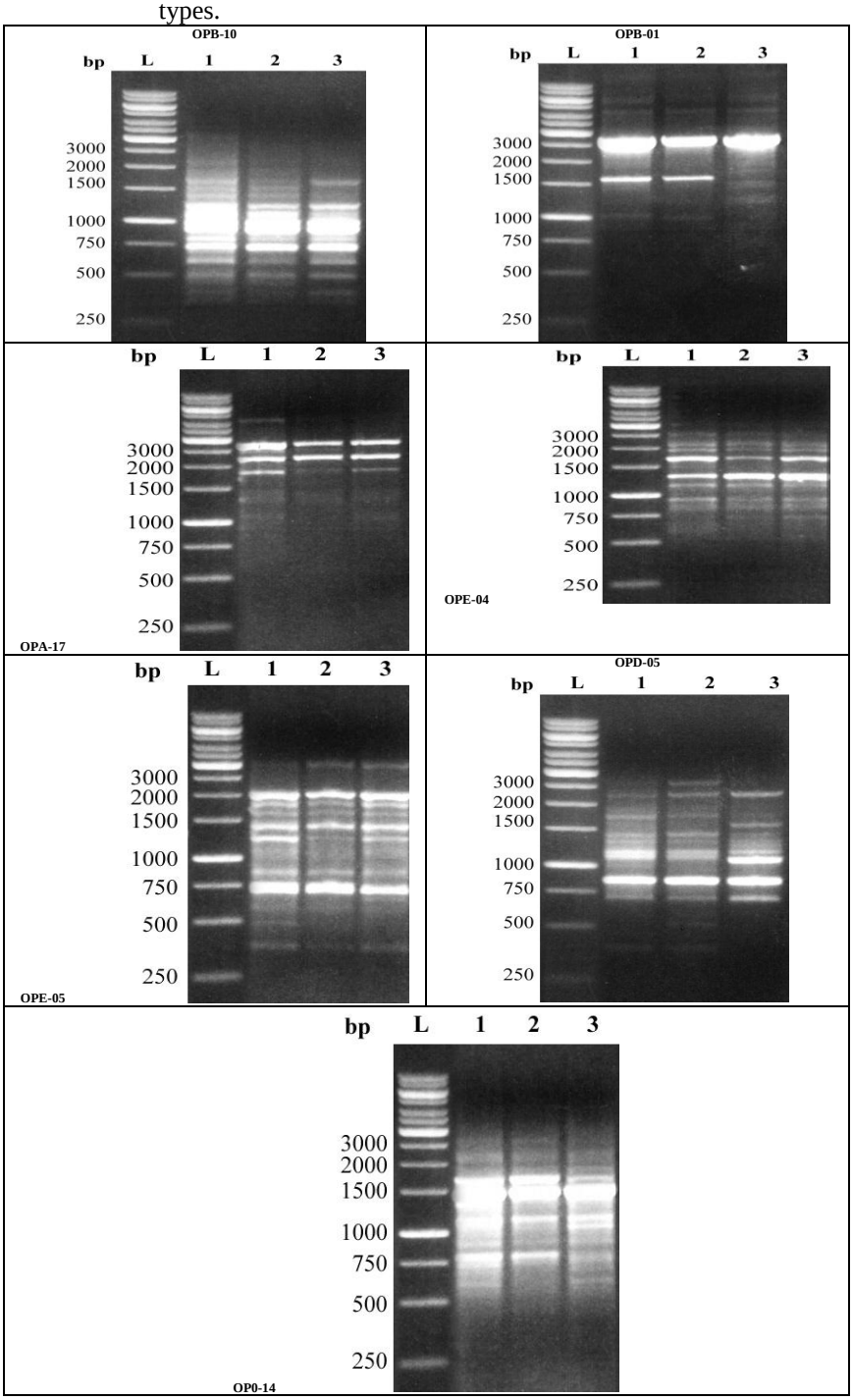
Table (10): Three cabbage genotypes characterized by unique positive and/or negative RAPD markers, marker size and total number of markers.

Primer	B.M.		B.		Br.		Positive marker	Negative marker	Total markers
	Positive marker	Negative marker	Positive marker	Negative marker	Positive marker	Negative marker			
OPE-04	1580	736					1	1	2
OPE-05	457				608	553	2	1	3
OPO-14				580	2007 1028	2672	2	2	4
OPA-17						3915	0	1	1
OPB-10	2346					1642	1	1	2
OPD-05			2803 471		1642 1051	1099	4	1	5
OPB-01	2052	1257			1570	1642 1005	2	3	5
Total	4	2	2	1	6	7	12	10	
	6		3		13		22		

Table (11): Genetic similarity values of three cabbage genotypes calculated from 39 DNA fragments generated with seven primers

Similarity matrix	**1**	**2**	**3**
1	1		
2	0.923	1	
3	0.825	0.85	1

Fig. (2): Agarose gel electrophoresis of RAPD profile of three cabbage genotypes.



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فحص الاختلافات الوراثيه لبعض الطرز الوراثيه لكل من البروكولى و الكرنب بإستخدام طريقه RAPD .

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تم استخدام عشرة واسمات (markers) لتحديد الاختلاف و العلاقه الوراثيه بين اربعه تراكيب وراثيه من البروكولى و ثلاث تراكيب وراثيه من الكرنب.
أظهرت نتائج تحليل ال RAPD ان كل الخمسه واسمات التى تم استخدامها و الخاصه بمحصول البروكولى نتج عنها أشكال مظهريه متعدده فى جميع التراكيب الوراثيه المختبره.
كذلك اظهرت النتائج وجود 39 حزمه ناتجه من جميع التراكيب الوراثيه للبروكولى و ان 21 واسمه أعطت أشكال مظهريه متعدده بنسبه 85.53 % و كانت نسبه 46.15 % حزمه مشتركه ما بين الاربعه تراكيب وراثيه.
و من ناحيه أخرى قد أظهرت السبعه واسمات المستخدمه للتفريق بين الثلاث تراكيب وراثيه للكرنب وجود 69 حزمه ناتجه من جميع التراكيب الوراثيه للكرنب و ان 23 واسمه أعطت أشكال مظهريه متعدده بنسب 33.33 و كانت نسبه 66.67 حزمه مشتركه ما بين الثلاث تراكيب وراثيه للكرنب.
و أخيرا فقد أظهرت هذه الدراسة عموما الى إمكانية استخدام طريقه ال RAPD كوسيلة ذات قدرة على التفريق ما بين التراكيب الوراثية لكل من الكرنب و البروكولى.