EVALUATION OF GENETIC DIVERSITY OF SOME *BRASSICACEAE* IN EGYPT ASSESSED BY RAPD AND PROTEIN MARKERS

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ABSTRACT

The *Cruciferae* = *Brassicaceae* family have large number of species with a major economic, medicinal importance and a great genetic diversity in the flora of Egypt .The genetic analysis was carried out on 11 Egyptian samples of *Brassicaceae* ollected from different localities to detect the genetic variability, to conserve these species and to facilitate breeding program. Polyacrylamide gel electrophoresis (SDS-PAGE) and RAPD and were used to characterize those species. SDS-protein electrophoresis of total proteins showed a total number of 12 protein bands were observed among the species studied. *Eruca sativa* collected from Burg El-Arab and North Sinai were found to have the highest number of 10 bands, while the lowest number of 7 bands was observed in the sample of *Capsella*, *Anastatica* , *Brassica*, *Matthiola* and *Zilla* genera. The highest molecular weight protein band (70 KDa) among the studied samples was recorded in *Eruca sativa*, while the lowest one (18 KDa) was detected in the five species of the genera *Capsella*, *Anastatica* , *Brassica*, *Matthiola* and *Zilla*. Bands of molecular weight (70 and 67) could be considered as species specific bands due to its presence in the two regions of *Eruca sativa*, Moreover, six bands of the molecular weight 112, 37, 30, 24, 22 and 20 KDa were considered as monomrphic bands, indicate the relationship among these species of the same family *Brassicaceae*. A total of 68 amplification products were obtained using 6 preselected random amplified polymorphic

DNA (RAPD) primers. Eighteen monomorphic bands and 48 polymorphic bands (70.5% of polymorphism) were revealed with kit A,B and D. RAPDs revealed a significant level of polymorphism among the accessions.. The results indicate the occurrence of a considerable genetic variation and diversity among *Brassicaceae* species.The overall dendrograms based on SDS-PAGE and RAPD clearly indicated that there was correlation and diversity among the eleven samples of *Brassicaceae* species by three clusters, *Eruca sativa* two locations, *Diplotaxis harra* ,*Diplotaxis acris* and *Raphanus sativums* clustered together ,while *Brassica nigra* and *B. alba* clustered together, also *Zilla spinosa* , *Matthiola inaca* ,*Capsella brusa* and *Anestatica heronticum* shared third cluster.

Key word: *Brassicaceae*, SDS-PAGE, RAPD -PCR, genetic polymorphism

INTRODUCTION

Brassicaceae are the largest family of the Brassicales order. It is a natural family of major economic importance, Mabberley (1997) reported the number of genera to be 365 and the number of species to be 3250. On the other hand, Judd *et al*. (1999) recorded 419 genera and 4130 species belonging to this family. The *Cruciferae* is classified into 13 tribes, *Sisymbrieae*, *Arabideae*, *Lepidieae* and *Brassiceae*. Only two of the tribes, the *Brassiceae* and *Lepidieae*, can be regarded as natural, which are confined to South Africa. In the flora of Egypt *Cruciferae* is well represented. Teckholm (1974) reported 61 genera and 106 species distributed in the different habitat types of the country. On the other hand, El-Hadidi and Fayed (1995) recorded 55 genera and 108 species for the family. Most members of *Cruciferae* are food or ornamental plants, e.g. *Raphanus sativus* L., *Brassica rapa*, *Brassica oleracea* (L.) var. capitata. Very few e.g. mustard and *Brassica nigra* L. are of medicinal value. Mustard plants or their oils are included in ointments of rheumatic pains. It is used as emetic in cases of poisoning. It may be used as stimulant for the heart (Naim *et al*., 1984). *Brassica* are major oil crop as well as broccoli, cabbage and mustard are increasly important part of human diet world wide. In general, genetic improvement of crops can be accelerated when broad genetic diversity and the information of these genetic resources are available. Research on *Brassica* germplasm could enhance the edible

oil production and nutritional benefits of these plants. The collection of these genetic resources and the assessment of genetic diversity within and between species should have the priority important and attention. At the same time it is necessary to develop better methods of characterization and evaluation of germplasm to improve strategies for conservation and collection of germplasm and to increase utilization of plant genetic resources .Exploitation of genetic variability is of major importance in basic genetic studies and in plant improvement programs. Traditionally, markers based on morphological differences among individuals have been used to demonstrate the genetic variability. With the development of electrophoretic techniques, (SDS-PAGE) variations offered a significant improvement to measure and characterize the genetic variation in and among population of many crops (Nxomani *et al*., 1994). The Biochemical (SDS- PAGE) are the cheapest and simplest methods that offer sufficient information for use in practical plant breeding and serve as a starting point for DNA-based studies (Popov *et al*., 2002). Seed protein is highly stable, being unaffected by environmental conditions Badr *et al* 1998 and Badr *et al* 2000). Thus electrophoretic banding patterns of total protein as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) have provided a valid source of taxonomic evidences and were used to address taxonomic relationships at the generic and specific levels and to investigate genetic variations, for example *Trifolium* (Badr, 1995) and *Hordeum vulgare* (El-Rabey *et al*., 2002). Sanches-Yelamo *et al.* (1992) carried out a comparative electrophoretic study of leaf proteins in 15 wild taxa of the genera Diplotaxais, Erucastrum and Brassica (*Cruciferae*). Their results supported the close affinity among the species of each genus. However, the information on SDS-PAGE on different species of *Brassicaceae* for genetic diversity is still limited. However, the advantage of molecular biology techniques has provided more powerful markers that allow the visualization of polymorphisms directly at the DNA level and proved extremely effective tools for distinguishing between closely related genotypes. Since the mid 1980s, genome identification and selection has progressed rapidly with the help of PCR technology .A large number of marker protocols that are rapid and require only small quantities of DNA have been developed . Each marker technique has its own advantages and

disadvantages. Among molecular markers, random amplified polymorphic DNA (RAPD) has been employed in genetic research owing to their speed and simplicity .(Welsh and McClelland 1990) indicated that the main advantages of the RAPD technology included its suitability for work on anonymous genomes, applicability to problems where only limited quantities of DNA are available, efficiency and low expense Williams *et al.* (1990), more efficient in determining the genetic relationships between different related species (Demeke *et al*. 1992, Thormann *et al*. 1994, Ren *et al*. 1995), good for the identification of cultivars (Hu and Quiros 1991 and Divaret and Thomas 1998). Although the use of RAPD technique for the study of genetic variation has been demonstrated as suitable in many species, less attention has been actually devoted to the selection of the most suitable approaches for genetic distance coefficients calculation. In this study a survey of leaf protein was carried out to asses the protein polymorphism among different species of *Brassicaceae* to investigate the electrophoretic banding pattern and to clarify the genetic nature of polymorphic bands ,protein analysis (SDS-PAGE) and RAPD were carried out on 11 taxa of the *Cruciferae* collected from their natural habitats from the flora of Egypt from different localities and to assess the diversity among them .The data obtained were analyzed by the SPSS program using the UPGMA clustering method to characterize the taxa studied and find out the relationship and diversity among them.

MATERIALS AND METHODS

Materials of the 11 taxa were collected from various habitats in Egypt. The studied species, the localities from which they were collected are given in Table 1.

1- Extraction of total protein .

Bulked leaf sample (0.25)gm of each sample was ground with liquid nitrogen and mixed with extraction buffer pH7.5 (50 mM tris, 5% glycerol and 14 mMB- mercapto ethanol) in a morter with pestle, left overnight then vortexed for 15 sec and centrifuged at 10.000 rpm at 4^0c for 10 min. The supernatants were transferred to new eppendorf tubes and kept in freeze until use for electrophoresis analysis. Gel was stained with commassie brilliant blue solutions for the protein detection.

2- DNA extraction.

Genomic DNA was extracted from fresh young leaves of ten plants chosen randomlyfor each population by CTAB method of Doyle and Doyle (1987). Leaves of eleven plants from a single population were bulked prior to extraction. DNA was quantitated by spectrophotometer and gel electrophoresis.

2-1- RAPD for DNA amplification

RAPD reactions were conducted using twenty arbitrary 10-mer primers according to Williams *et al.* (1990).The reaction conditions were optimized and mixtures were prepared (25 ul total volumes) consisting of the following: dNTPs(8mM)1.0ul, Taq DNA polymerase(5U/ul), 0.2ul 10X buffer with 15mM MgC12 ,2.5ul Primer , Template DNA(10-50 ng/ul) 1.0 ul, H20 (dd) 19.3 ul. The most reproducible primers were OPB1, OPB2, OPB5, OPB6, OPD1, OPA3.The nucleotide sequences of these primers are present in Table (5). Amplification was carried out in Strategene Robocycler Gradient 96 which was programmed for 40 cycles as follows: Denaturation (one cycle) 94°C for 4 minutes, (40 cycles) of the following sequence 94°C for 1 minute and 30 second, 36°C for l minute and 30 second, 72° C for 2 minutes and 30 second then extension (one cycle) 72 ° C for 7 minutes. Amplification products were analyzed by electrophoresis on 1.2% agarose gels using TBE buffer and then stained with ethidium bromide. A molecular marker DNA Ladder was used. The run was performed for one hour at 100 volt. Bands were detected on UV-transilluminator and photographed by Gel documentation system.

RESULTS AND DISCUSSION

1. SDS-PAGE leaf proteins:

Leaf protein analysis was carried out on 11 taxa of 10 species collected from the flora of Egypt from different localities (Table 1). The leaf protein banding profiles of the 11 taxa are illustrated in Fig.1. A total number of 12 protein bands were observed among the species studied. *Eruca sativa* collected from Burg El-Arab and North Sinai were found to have the highest number of 10 bands, while the lowest number of 7 bands was detected in the five species of the genera *Capsella*, *Anastatica* , *Brassica*, *Matthiola* and *Zilla*. Bands of

molecular weight (70 and 67) scould be considered as species specific bands due to its presence in the two regions of *Eruca sativa*, also the bands recorded at the molecular weights of 40 and 45 KDa distinguished *Eruca sativa* , *Dplotaxis harra* and *Diplotaxi acris* species. Moreover, six bands of the molecular weight 112, 37, 30, 24, 22 and 20 KDa were considered as monomrphic bands, indicate the relationship among these species of the same family *Brassicaceae*.

The present investigation is in agreement with the study of Sanches *et al*. (1992). This study supports the close affinity among the species of each genus. Leaf protein banding patterns as revealed by SDS-PAGE produces reproducible band pattern (profile) when proteins are prepared in a standard method and hence have valid value in taxonomic purposes. Consequently, proteins with identical electrophoretic mobility are deemed to represent the same unit character. Therefore characters derived from seed proteins have been utilized in plant taxonomy at different levels to construct phenetic classifications (Boulter, 1981; Smith, 1984, Echeverrigaray *et al*., 1998, El-Fiky *et al* .,2002 and Raham and Yutaka 2004).

Based on protein marker, similarity matrix was developed by SPSS computer package system in Table (5). The closest relationship was scored between *Eruca sativa* from two locations with similarity %99.9. While *Capsella prusa* and *Eruca sativa* gave the lowest similarity of 10% which were considered distantly related and not closely related species. The dendrogram based on SDSPAGE separated the ten species into two main clusters. Fig (3).

2.RAPD –PCR

Six preselected RAPD primers were used in the present study to identify the eleven samples of *Brassicaceae* as shown in Tables (3 and 4) Fig(2) . Eighteen monomorphic bands and 48 polymorphic bands (70.5% of polymorphism) were revealed with kit A,B and D . In general, the results indicated that RAPD markers gave adequate distinctions among eleven *Brassicaceae* samples. These results were in partial agreements with the finding of Abdel-Tawab *et al* .(1998) who identified nine sugarcane cultivars using RAPD markers. Moreover, Cheng *et al* .(2000) investigated the genetic variability of *Astragalus* medicinal materials in Taiwan using RAPD analysis .Also, these result agreed with those of EL-Saied *et al*. (2004) tested eight *Acacia* species using RAPD markers and indicated that RAPD

technique gave remarkable molecular discrimination between the eight *Acacia* species. Hassan (2005) who reported that RAPD markers were one of the best choice for the evaluation of diversity and assessing the genetic relationships between *moringa* and *mentha* genotypes with high accuracy . Moreover, Said (2005) tested five individual plants from *caper* and *arghel* species using thirteen preselected random primers . They reported that it was possible to differentiate between individual plants of the same species using RAPD markers.

Moreover, Liu *et al* (2007) employed randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) for germplasm identification and genetic diversity analysis of 17 radish (*Brassicaceae)* accessions. The RAPD marker was the most polymorphic (93.4%) among the three marker types. Also, Nisar *et al* (2007) and Hu *et al* (2007) evaluated the genetic diversity in 20 rape lines (10 entries each of *B. napus* and *B. campestris*) from Pakistan by random amplified polymorphic DNA (RAPD), indicated that genotypes of *B. napus*, and *B. campestris* were genetically apart from the other lines. Park *et al* (2007) characterized 24 leaf mustard (*Brassica juncea*) cultivars, the genetic relationship and DNA polymorphism by using Random amplified polymorphic DNA (RAPD) marker, they selected four random primers formed 12 polymorphic bands and Separated 24 accessions into 5 groups based on UPGMA cluster analysis resulted that the very close relationship in the quantitative character analysis did not agree with the RAPD analysis and quantitative analysis.

2.1.Species –specific – RAPD markers:

Some specific markers for some *Brassicaceae* species RAPD analysis are listed in table (4). Ten out of 128 RAPD markers were found to be species specific .These markers were scored for the presence of unique bands for a given species. *B.alba* had two unique bands could be scored by OPB2 and OPB6.*Matthiola inaca* and *Raphanus sativum* each could bedistinguished by species specific band by OPB5. *Anastatica herontica* , *Capsella brusa* and *Matthiola inaca* could bedistinguished by species specific band by OPD1 (Afiah et al .2007)

Based on RAPD marker, similarity matrix was developed by SPSS computer package system in Table (6). The closest relationship

was scored between *Diplotaxis harra* and *Raphanus sativum* with similarity %99.9. While *Capsella brusa* and *Eruca sativa* gave the lowest similarity of 00.9% which were considered distantly related and not closely related species. The dendrogram based on RAPD separated the eleven samples into two main clusters. Moreover, *Eruca sativa* from two locations was separated in subcluster and *Diplotaxis acris*, *Diplotaxis harra*and *Raphanus sativum* were clustered together in another subcluster but in the same cluster with *Eruca sativa* from two locations. While remain species clustered in the second cluster as shown in Fig(4)

Table (1) : Taxa of *Brassicaceae* samples.

No	Taxa	Source
1	*Eruca sativa*	Burg EL -Arab
2	*Eruca sativa*	North Sinai
3	*Diplotaxis acris*	Cairo- Suez Desert Road
4	*Diplotaxis harra*	Cairo- Suez Desert Road
5	*Raphanus sativum*	Burg EL- Arab
6	*Capsella- brusa*	Burg EL- Arab
7	*Anastatica herontica*	Cairo- Suez Desert Road
8	*Brassica alba*	Cairo- Alex. Desert Road
9	*Matthiola inaca*	Burg EL- Arab
10	*Zilla spinosa*	Cairo- Alex. Desert Road
11	*Brassica nigra*	Cairo- Alex. Desert Road

Table(2): Protein banding pattern of the studied *Brassicaceae* species as revealed by SDS-PAGE for total protein

M W KD	1	2	3	4	5	6	7	8	9	10	11
112	1	1	1	1	1	1	1	1	1	1	1
100	0	0	0	0	0	0	0	0	0	0	1
70	1	1	0	0	0	0	0	0	0	0	0
67	1	1	0	0	0	0	0	0	0	0	0
45	1	1	1	1	1	0	0	0	0	0	0
40	1	1	1	1	1	0	0	0	0	0	0
37	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1
18	0	0	0	0	1	1	1	1	1	1	0

The presence of band=1 the absence= 0 shading= common band

Code numbers (1-15) represent the fifteen ***Brassicaceae*** species as explained in table (1).

Table (3): Nucleotide sequences of RAPD primers among *Brassicaceae* species.

Primer Code NO.	Primer Sequences
OPB1	5'-GTTTCGCTCC-3'
OPB2	5'-TGATCCCTGG-3'
OPB5	5'-TGCGCCCTTC-3'
OPB6	5'-TGCTCTGCCC-3'
OPD1	5'-TCTGGTGAGG-3'
OPA3	5'-AGTCAGCCAC-3'

Table (4): RAPD primers species - specific marker for *Brassicaceae* species.

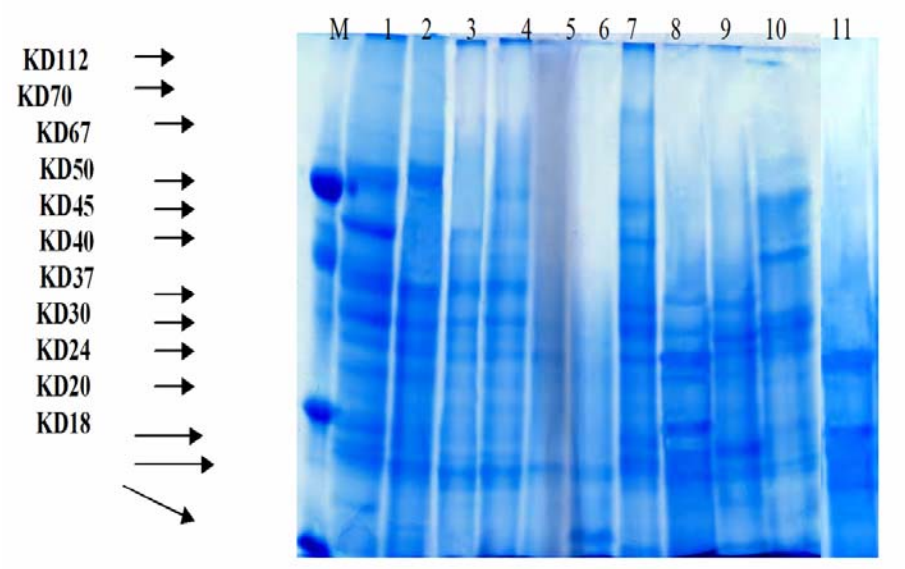
Primer Code NO.	*Plant species*	*Unique* bands
OPB1	*Brassica nigra*	1
OPB2	*Brassica alba*	1
OPB5	*Matthiola inaca*	1
OPB5	*Raphanus sativum*	1
OPB6	*Brassica alba*	1
OPD1	*Anastatica heronticum*	1
OPD1	*Matthiola inaca*	1
OPD1	*Capsella brusa*	2
OPA3	*Brassica alba*	1
TOTAL		**10**

Table (5): results of SDSPAGE for the eleven *Brassicaceae* samples.

	1	2	3	4	5	6	7	8	9	10	11
1	.000										
2	.999	.000									
3	.999	.999	.000								
4	.999	.969	.999	.000							
5	.992	.969	.923	.999	.000						
6	.190	.105	.100	.109	.100	.000					
7	.100	.105	.100	.109	.100	.100	.000				
8	.154	.131	.105	.100	.102	.100	.109	.000			
9	.154	.131	.105	.100	.102	.100	.109	.100	.000		
10	.131	.108	.108	.105	.385	.100	.100	.923	.923	.000	
11	.254	.231	.200	.100	.262	.299	.222	.999	.999	.923	0.00

Table (6):Amplification results of the six RAPD primers for the eleven *Brassicaceae* sample

	1	2	3	4	5	6	7	8	9	10	11
1	.000										
2	.923	.000									
3	.769	.692	.000								
4	.692	.769	.923	.000							
5	.692	.769	.923	.999	.000						
6	.154	.231	.385	.462	.462	.000					
7	.009	.077	.231	.308	.308	.846	.000				
8	.154	.231	.385	.462	.462	.999	.846	.000			
9	.154	.231	.385	.462	.462	.999	.846	.999	.000		
10	.231	.308	.308	.385	.385	.923	.769	.923	.923	.000	
11	.154	.231	.385	.462	.462	.999	.846	.999	.999	.923	0.00



Figure(1): SDS-PAGE profile of leaf total-protein among the fifteen Brassicaceae species M= Protein marker , KD= Kilo Dalton.

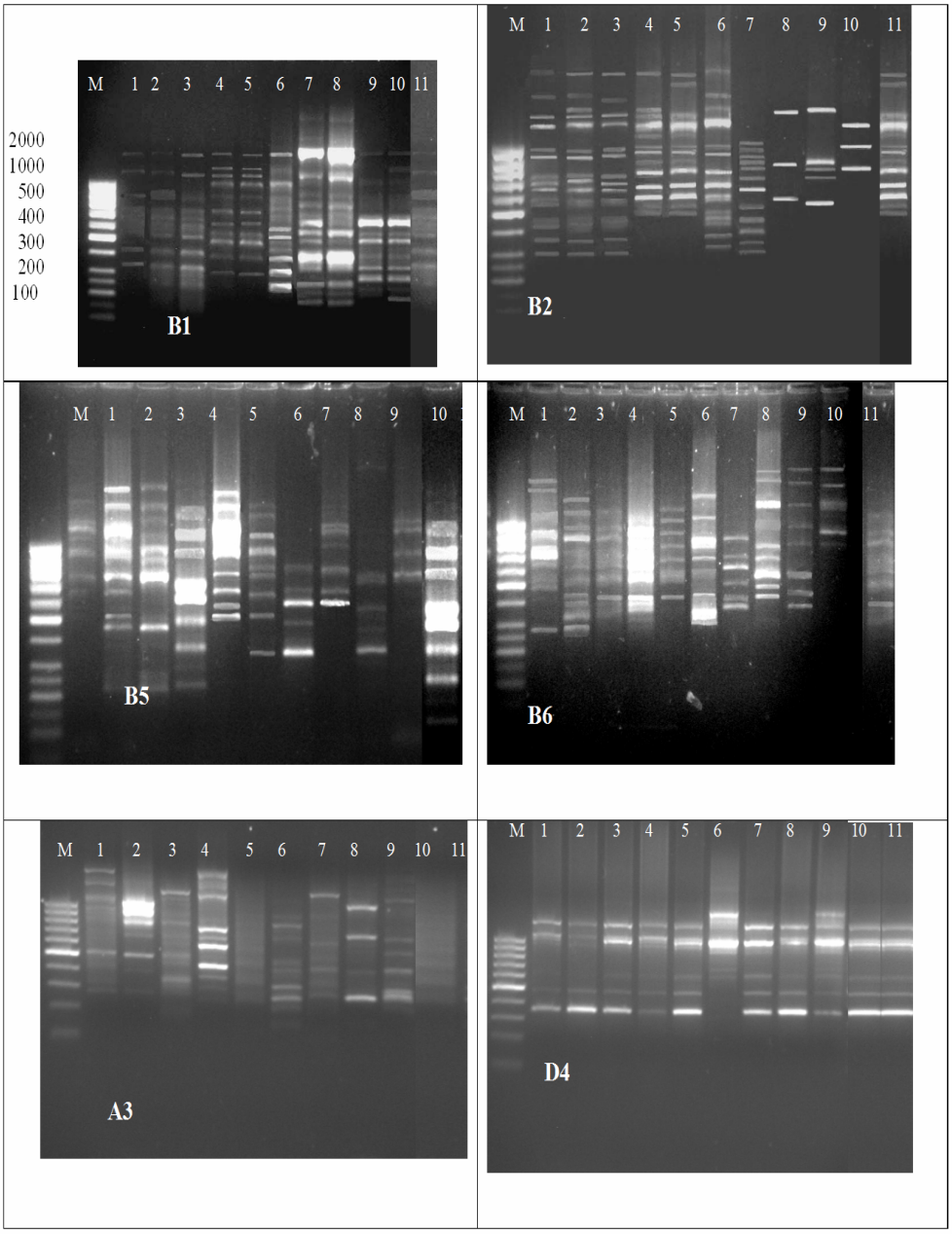


Fig (2): DNA polymorphism using RAPD with primers of OPB1, OPB2, OPB5, OPB6, OPD1and OPA3 in the eleven samples of Brassicaceae .

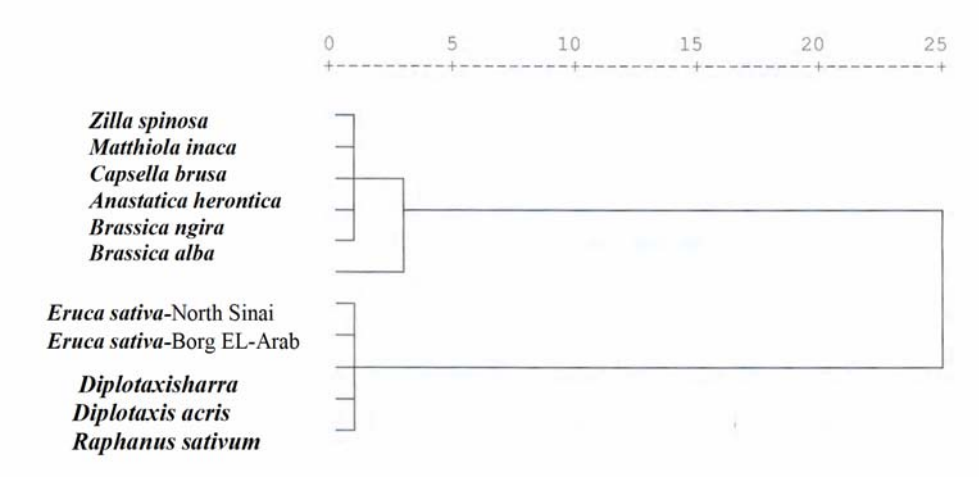


Fig (3): Dendrogram based on isozyme of Brassicacceae species

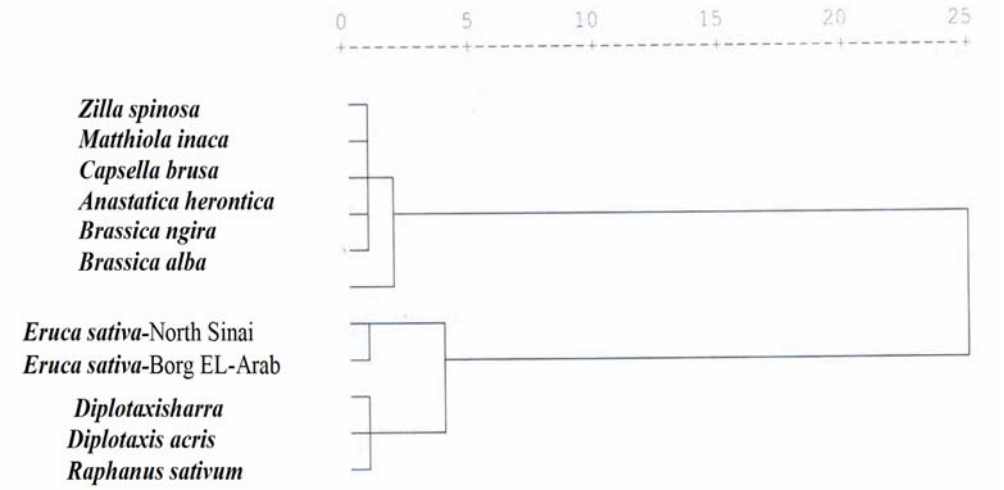


Fig (3): Dendrogram based on RAPD of Brassicacceae species

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تقييم التنوع الوراثي فىبعض نباتات العائلة الصليبية فىمصر باستخدام البروتين والتضخيم العشوائى المتعدد للDNA

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تضم العائلة الصليبية العديد من الانواع النباتيه ذات القيمة الاقتصادية والاهمية الطبية والتنوع الكبير الوراثى فى الفلورا المصرية. تم اجراء التحليل الوراثى لاحدى عشرة عينة تتبع العائلة الصليبية وتمثل احدى عشرعينة ممثلة لانواع نباتية مختلفة من اماكن مختلفة بمصر لمعرفة الاختلافات الوراثية وكوسيلة لحفظها وتسهيل برامج التربية الانتخابية،وذلك باستخدام التفريد الكهربى للبروتينات وتقنية (RAPD) (DNA) ،اظهر التفريد الكهربى للبروتينات الكلية اثنى عشر شريطا (حزم) بروتينية وتميز الجرجير البرى ببرج العرب وشمال سيناء باعلى عدد منها عشرة شرائط (حزم) بروتينية بينما وجد اقلهم سبعة (حزم) بروتينية فى خمسة اجناس، مخلة الراعى ، كف مريم ، الخردل، المنثور و سلة الجمال ، كما تميز الجرجير البرى بشرائط رئيسية ذات الاوزان الجزيئية 70، 67 كيلودالتون التى تعتبر مميزة للنوع ، بينما تميز الاجناس الخمسة السابقة بوجود اقل الشرائط وزنا جزيئيا 18 كيلودالتون ، ستة شرائط وحيدة المظهر من الاثنى عشر باوزان جزيئية 112، 37، 30، 24، 22 ، 20 كيلو دالتون ، مما يؤكد انتمائها للعائلة الصليبية ،بالاضافة للحصول على ثمانى مئةوستون حزمة باستخدام ستة بادئات عشوائية فى تكنيك التعدد المظهرى للتضخيم العشوائى لDNA RAPD ،واخيرا كانت تقنية RAPD اكثر توضيحا وتفرقتا من التفريد الكهربى للبروتينات الكلية ، بين النباتات باستخدام ستة بائات عشوائية لتمييز بين الاحدى عشر عينة ، كما اوضح الدندروجرام الناتج منRAPD حزمتين رئيسيتين ، الاولى للجرجير البرى بموقعيه والفجل البرى بانواعه الثلاثة والثانية للخردل الابيض والاسود بالاضافة الى الخمسة انواع الباقية من سلة الجمل ، المنثور ،كف مريم ، مخلة الراعى.