GENETIC POLYMORPHISM OF SOME MEDICINAL PLANTS BELONGING TO *Brassicaceae* USING MOLECULAR MARKERS

RASHA M. A. KHALIL[1], KH. A. SOLIMAN[2], NAHED A. K. F. RASHED[1] AND S. A. IBRAHIM[2]

1. *Plant Genetic Resources Dept., Desert Research Center, Matariya, Cairo, Egypt*
2. *Dept. of Genetics, faculty of Agriculture, Ain Shams Univ., Cairo, Egypt*

Exploitation of genetic variability is of major importance in basic genetic studies and in plant improvement programs. The Cruciferae (*Brassicaceae*) is the largest family of the Brassicales order. It is a natural family of major economic importance. The number of genera about 365 and the number of species about 3250, Mabberley (1997) and Judd *et al*. (1999) recorded 419 genera and 4130 species belonging to this family and is classified into 13 tribes. In Egypt, Teckholm (1974) recorded 61 genera and 106 species distributed in different habitat types. On the other hand, El-Hadidi and Fayed (1995) recorded 55 genera and 108 species for it.

Mustard plants or their oils can heal rheumatic pains and as emetic in cases of poisoning. It may be used as stimulant for the heart (Naim *et al*., 1984). *Brassica* are major oil crop and broccoli, cabbage and mustard are important part of human diet worldwide. In general, genetic improvement of crops can be accelerated when broad genetic diversity and the information of these genetic resources are available. The collection of these genetic resources and the assessment of genetic diversity within and between species should have high priority. Traditionally, markers based on morphological differences among individuals have been used to demonstrate the genetic variability, but with the development of electrophoretic techniques, the biochemical analysis become the cheapest and simplest methods that offer sufficient information to use in plant breeding and serve as a starting point for DNA-based studies (Popov *et al*., 2002). Moreover, the best molecular markers for genome mapping, marker assisted selection, phylogenetic studied, and crop conservation has low cost and labor requirements and high reliability is called inter simple sequence repeat (ISSR), has been available (Zietkiewicz *et al*., 1994). Microsatellites are very short (usually 10-20 base pair) stretches of DNA randomly and widely distributed along the genome and they can be analyzed efficiently by the polymerase chain reaction (PCR), using specific primers (forward and reverse) to their flanking regions. The variation in (PCR) product length is a function of the number of SSR units. Primers based on a repeat sequence, such as (CA)n, can be made with a degenerate 3'-anchor, such as (CA)

8 RG or (AGC) 6 TY (Tsumara *et al*., 1996; Nagaoka *et al*., 1997). ISSR markers were successfully used for estimating the genetic diversity in several crops, for instance barley (Afiah *et al*., 2007a) and canola (Afiah *et al*., 2007b; Afiah and Farag, 2008). In this study isozyme and ISSR were carried out on 11 ecotypes of the Cruciferae collected from different localities of Egypt and to assess the genetic diversity among them.

MATERIALS AND METHODS

Materials of the 11 taxa were collected from various habitats in Egypt (Table 1).

1- Extraction of total proteins

Bulked leaf sample (0.25) g of each ecotype 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°C for 10 min. The supernatants were transferred to new eppendorf tubes and kept at -20°C until use for electrophoresis analysis.

1-1- Isozyme detection

The gels were stained for the isozymes detection according to the enzyme system and incubated at 37°C in dark for complete staining after adding the appropriate substrate and staining solutions (Wendle and Weeden, 1990).

2- DNA extraction

Genomic DNA was extracted from fresh young leaves of ten plants chosen randomly for 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 quantified by spectrophotometer and gel electrophoresis.

2-2- ISSR -PCR

ISSR-PCR reactions were conducted using nine specific primers, for the nine genotypes as presented in Table (3). The reaction condition was optimized and mixturesconsisted of dNTPs (8mM mix), 2.5 µl Taq DNA polymerase (5U/ µl) 0.3 µl, 10 x buffer with 1.5 mM $MgCl_2$ 3.0 µl, primer (l0 mM) 2.0 µl, template DNA (50 ng/µl) 2.0 µl and H_2O (dd) up to 30 µl. Amplification was carried out in Stratgene Robocycler Gradient 96 which was programmed for 45 cycles as follows. Denaturation (one cycle) 94°C for 2 minutes, followed by 30 cycles as follows 94°C for 30 second, 44°C for 45 sec. 72°C for 1 minute and 30 sec. and finally one cycle extension at 72°C for 20 minutes, and 4°C (infinitive). 15 µl of PCR- products were resolved in 1.5°/o GTG agarose gel electrophoresis with 1x TAE running buffer. The run was performed at 80 V for 180 min and the gel was stained with ethidium bromide. A marker of 1 Kb plus DNA Ladder 1 µg /µl (Invitrogen) that contains a total of twenty bands ranging from 12000 to 100 bp was used. Bands were detected on UV-

transilluminator and photographed by Gel documentation system UVP2000.

RESULTS AND DISCUSSION

1. Isozyme analysis

Five isozyme systems including α and β Est (esterases), Prx (peroxidase), Acp (acid phosphatase), Adh (alchol dehydrogenase), Mdh (malate dehydrogenase) were used to test the genetic variability among the studied species (Fig. 1). As can be seen from the results (Table 2) a total of 26 bands were detected using the five isozyme systems, seven bands were monomrphic and the other 19 bands were polymorphic among all studied *Brassicaceae* species. Isozymes have been used successfully to evaluate genetic variability within and among *Brassicaceae* species (Ozaki *et al*., 2000; Sabu *et al*., 2001).

Esterases (Est), generally, α-esterase exhibited highly polymorphic patterns among the studied genotypes. Est is a gene family hydrolyze ester bond in lipid to produce plant energy for biochemical reactions, to allivate energy level for other metabolisms especially under drought conditions (Durate *et al*., 2007). Results indicated that esterase revealed a total of seven bands, four polymorphic bands (no. 4, 5, 6 and 7) and three monomorphic bands (no.1, 2 and 3). However, Kumar and Gupta (1985) ascertained that esteras patterns were successfully used to characterize different Indian mustard genotypes. Ahmed *et al*. (2003) detected genetic diversity for esterases in *Brassica* members and *Acacia*, which agreed with our results. Generally, β esterase demonstrated a highly polymorphism among the studied species. Results indicated that this pattern revealed a total of seven bands. The first and second bands were monomorphic, while the other six bands were polymorphic.

Moreover, Acid phosphatase (Acph) patterns of all studied samples revealed a high polymorphism in band's number among the studied samples, four bands (2, 4, 5 and 6) were polymorphic, and two bands (1 and 3) were monomorphic. Tso and Chen (1997) results agreed with our results and indicated that acid phosphatase is dimeric isozyme which universally occurs in the leaves, stems, and roots of most plants. In *Brassicaceae*, it controls the transformation of qiunones as secondary compounds.

In addition, peroxidase patterns of all studied samples revealed a high polymorphism in band's number among the studied samples three bands (2, 4 and 5) were polymorphic while two were monomorphic bands (no.1 and 3). Our results agreed withthose of Mara *et al*. (1999) in sunflower and Lagrimini and Rothstein (1987) in tobacco who concluded that peroxidases catalyze H_2O_2 dependent oxidation of a wide variety of substrates like phenolic residues into cell wall polymers as monomeric isozyme. Lignification, auxin metabolism, stress

response and defense against pathogens. (Welinder, 1992; Schuller *et al*., 1996).

Malate dehydrogenase (Mdh) exists in the mitochondria and cytoplasm. It catalyzes the reversible oxidation of malate to oxaloacetate. Thus, it plays a major role in central metabolism. Mdh banding patterns gave a total of two bands as monomorphic bands. The result agrees with Mark and William (1989) and Duart *et al*. (2007) who indicated that Mdh in *Cactaceae* and *Broccoli* is a dimeric enzyme of three bands with two or three loci.

Alcohol dehydrogenase (Adh) remove two hydrogen atoms from the substrate and passes them to a suitable coenzyme acceptor like NAD+ or NADP+ in reverse reactions. The banding patterns of a total of two bands were monomorphic for the studied samples. The results agreed with these of Mukhlesur *et al*. (2005) who concluded that Adh gene family has two or three loci in angiosperm species and multiple loci in monocots.

Based on isozyme 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 of 99.9%. While *Raphanus sativum* and *Eruca sativa* gave the lowest similarity of 17% which were considered distantly related species. The dendrogram based on cellective isozyme pattern separated the ten species into two main clusters. Moreover, *Eruca sativa* from two locations, *Diplotaxis acris*, *Diplotaxis harraand* and *Raphanus sativum* were clustered together. While the remaining species were clustered in the second cluster as shown in Fig. (3).

2. ISSR analysis

Fourteen preselected ISSR primers were used in the present study to identify eleven species of *Brassicaceae* as shown in Tables (3 and 4) and Fig. (2). Fifty two monomorphic and 71 polymorphic distinct fragments (57.7% polymorphism) were revealed in the eleven tested species with these primers. The results showed that primers A17898, IS-O5, IS-O12 and B17898 were highly polymorphic (75% and 85.7% polymorphism). Moreover, primers B17899, HB12, IS-O2, IS-O6, IS-15 and IS-O4 were moderately polymorphic (from 50% to 66.6% polymorphism). On the other hand, primers IS-O1, HB10, A17899 and HB15 showed the lowest polymorphism (from 25% to 44.4%). The overall results of fourteen primers were illustrated in Table (3). Primers IS-O4, IS-O5, HB12, HB15, IS-O6, B17899, A17899 and A17898 gave the highest total number of bands from 9 to 12 bands. While, primers ISO-1, B17898, B17899, HB10, IS-O2, and IS-O15 gave the lowest total number of bands from 6 to 8 bands. In general, the result indicated that ISSR markers gave adequate distinction among *Brassicaceae* species.

Species-specific markers

Some specific markers for some *Brassicaceae* species across ISSR

analysis are listed in Table (4). Eight out of 123 ISSR markers were found to be species specific. These markers were scored for the presence of unique bands for a given species. *Brassica nigra* had two unique bands as revealed by A17898 and IS-O15. Then, the remaining species could be distinguished by one band for each marker for Anastatica herontica, Eruca sativa from Burg EL-Arab, D*iplotaxis acris, D. harra, Capsella brusa* and *Matthiola inaca*. Eight out of fourteen primers gave specific markers. The results agreed with those of Pharmawati *et al*. (2005) who reported that 17 out of 584 ISSR markers were found to be cultivar-specific; Hassan (2005) who found that 20 out of 110 ISSR markers were specific markers among ten genotypes of Moringa species using 11 ISSR primers and finally, Abdel-Tawab *et al*. (2007) who showed that 48 out of 164 ISSR markers were specific markers among seven genotypes of Mentha and Ocimum species using 10 ISSR primers.

Based on ISSR marker, similarity matrix was developed by SPSS computer package system in Table (6). The closest relationship was scored between *Diplotaxis harra* and *Diplotaxis acris* with similarity 99.9%. While *Capsella brusa* and *Diplotaxis acris* gave the lowest similarity of 50.3% which were considered distantly related species. The dendrogram based on ISSR separated the ten species into two main clusters. Moreover, *Eruca sativa* from two locations was separated in subcluster and *Diplotaxis acris*, while *Diplotaxis harra* and *Raphanus sativum* were clustered together. Another subcluster but in the same cluster included *Eruca sativa* from two locations (Samy *et al*., 2007). While remaining species were clustered in the second cluster as shown in Fig. (4).

Genetic relationships among Brassicaceae species

The closest relationship was scored between Diplotaxis acris and Diplotaxis harra with similarity of 98.9%, then Eruca sativa from two locations with similarity 98.6%. While *Eruca sativa* and *Brassica nigra* gave the lowest similarity of 9.5% which were considered distantly related species. The dendrograms based on isozyme and ISSR data (Fig. 5), clearly indicated that there was correlation between the eleven samples of *Brassicaceae* species by clustering them together and gave us another dimension to detect the genetic variability by showing the accurate differentiations and demonstrated significant levels of variation. In this study, the dendrogram separated *Eruca sativa* from two locations in subcluster, *Diplotaxis harra*, *Diplotaxis acris* and *Raphanus sativums* clustered together in another subcluster, on the other hand, *Brassica nigra* and *B. alba* clustered together and split away from *Eruca* and *Diplotaxis* genera into another second cluster, also *Zilla spinosa* and *Matthiola inaca* were observed to share a separate subcluster with Capsella brusa, Anestatica heronticum (Muminovic *et al*., 2005; Javidfar *et al*., 2006; Geng *et al*., 2007; Javidfar *et al*., 2007; Liu *et al*., 2007; Afiah *et al*., 2007b).

SUMMARY

The Cruciferae (*Brassicaceae*) family has large number of species with a major economic, medicinal importance in the flora of Egypt. The genetic analysis was carried out on 11 Egyptian ecotypes of *Brassicaceae* representing ten *Brassicaceae* species collected from different locations to detect genetic variability, to conserve these species and to facilitate breeding program. Isozymes and ISSR were used to characterize these species. Five isozyme systems including α and β Est (esterases), Prx (peroxidase), Acp (acid phosphatase), Adh (alchol dehydrogenase), Mdh (malate dehydrogenase) were used to test the biochemical genetic variability among the studied species, a total of 26 bands were detected using the five isozyme systems, 7 bands were monomrphic and the other 19 bands were polymorphic among all studied Brassicaceae species. Fifteen preselected ISSR primers were used in the present study to identify and evaluate genetic variability among *Brassicaceae* species .ISSR was more effective than isozymes in showing the diversity among the eleven ecotypes of *Brassicaceae* species. The overall dendrogram based on isozymes and ISSR clearly indicated that there were considerable diversity and relationships among the eleven ecotypes of *Brassicaceae* species by clustering the closely related ones together and gave us another dimension to detect the genetic variability in accurate differentiations and demonstrated significant levels of variation.

REFERENCES

Abdel-Tawab, F. M., Eman M. Fahmy, Hoda M. El-demerdash, O. M. Saleh, H. Fotouh and Gh. A. Gad EL-Karim (2007). Molecular phylogenetic relationships of two genera of *Labiatae* family. Egypt. J. Genet. Cytol., 36: 325-339.

Afiah, S. A., A. Z. E. Abdelsalam, E. A. Kamel, A. E. Dowidar and S. M. Ahmed (2007a). Molecular genetic studies on canola crosses under Maryout conditions. 8th African Crop Sci. Society a Conf., El-Minia, Egypt, 27-31 Oct., 633-642.

Afiah, S. A., K. Z. Ahmed and Kh. A. Soliman (2007b). Somaclonal variation in bread wheat (Triticum aestivum L.). 4-RAPD fingerprinting of elite genotype under Siwa Oasis conditions. 8th African Crop Sci. Society Conf., El-Minia, Egypt.

Ahmed, A. M., M. E. El-Saied, Farida M. and A. A. Morsy (2003). Biochemical Genetic Fingerprinting of eight acacia species from Egypt. Desert Ins. Bull., 53: 217-244.

Badr, A. (1995). Electrophoretic studies of seed proteins in relation to chromosomal oriteria and relationships of some taxa of trifolium. Taxson, 44: 183-191.

Doyle, J. J. and J. L. Doyle (1987). A rapid DNA isolation procedure for

small quantities of fresh leaf tissue. Phytochem. Bulletin, 19: 11-15.

Duarte, V. M. A., P. S. Garcia, A. B. E Garcia, J. R. Whitaker and C. T. Regalado (2007). Broccoli processing wastes as a source of peroxidase. Journal of Agricultural and Food Chemistry, 55: 10396-10404.

El-Hadidi, M. N. and A. A. Fayed (1995). (Eds): Materials from excursion flora of Egypt (EFE).

Geng, J. F., X. L. Hou, X.W. Zhang, Y. Cheng, Y. Li, Y. X. Yuan, W. S. Jiang, Y. P. Han and Q. Yao (2007). Construction of a genetic linkage map in non-heading Chinese cabbage (*Brassica campestris*) using a doubled haploid (DH) population. Journal of Nanjing Agricultural University, 30: 23-28.

Hassan, A. M. H. (2005). Identification of molecular marker for some morphological and biochemical characters in some medicinal plants unpublished M. Sc. Thesis, Ain Shams Univ., Fac. Agric.

Javidfar, F., V. Ripley, H. Zeinali, S. Abdmishani, A. A. S. Bushehri and R. T. Afshari (2007). Detection of molecular markers associated with linolenic acid content in spring oilseed rape (*Brassica napus* L). Agricultural Science Tabriz, 17: 47-56.

Jones C. J., K. J. Edwards, S. Castaglione, M. O. Winfield, F. Sale, C. Van de Wiel, G. Bredemeijer, M. Buiatti, E. Maestri, A. Malcevshi, N. Marmiroli, R. Aert, G. Volckaert, J. Rueda, R. Linacero, A. Vazquez and A. Karp (1997). Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol. Breed., 3: 381-390.

Judd, W. S., C. S. Campbell, E. A. Kellog and P. F. Stevens (1999): Plant Systematics. A Phylogenetic Approach Sinauer Associates, Inc. USA.

Kumar and Gupta (1985). Isozyme studies in Indian mustard (*Brassica juncea* L.). Theor. Appl. Genet., 70: 107-110.

Lagrimini, L. M. and S. Rothstein (1987). Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. Plant Physiology, 84: 438-442.

Liu, L. W., G. A. Liu, Y. Q. Gong, W. H. Dai, Y. Wang, F. M. Yu and Y. Y. Ren (2007). Evaluation of genetic purity of F_1 hybrid seeds in cabbage with RAPD, ISSR, SRAP, and SSR markers. Hort. Science, 42: 724-727.

Liu, P. W. and G. S. Yang (2004). Analyses of the genetic diversity of resynthesized *Brassica napus* by

RAPD and ISSR molecular markers. Acta Agronomica Sinica, 30: 1266-1273.

Mabberley, D. J. (1997). The plant-book, a portable dictionary of the vascular plants. Cambridge Univ. press.

Mara, D., M. Groppa, L. Tomaro and E. Marcelo (1999). Activity and expression of peroxidases from sunflower: Effect of development. Revista Brasileira de Fisiologia Vegetal, 11: 55-59.

Mark, R. H. and C. William (1989). Electrophoretic Band Patterns of Phospho glucoisomerase and Malate Dehydrogenase in the Prickly Pear Cactus (Cactaceae) from Southwestern Oklahoma. Proc. Okla. Acad. Sci., 69: 5-9.

Mukhlesur, R. M. and Y. Hirata (2005): Identification of different species and cultivars of *Brassica* by SDS-PAGE, isozyme and molecular marker. J. Plant Biotech., 7: 27-35.

Muminovic, J., A. Merz, A. E. Melchingeraa and T. Lubberstedt (2005). Genetic structure and diversity among radish varieties as inferred from AFLP and ISSR analyses. Journal of the American Society for Horticultural Science, 130: 79-87.

Naim, M. S., M. El-Shafey, H. Sharoubeem and A. El-Bayoumi (1984). Botany, Dar Al-Maaref, Cairo, Egypt, pp. 330.

Ozaki, Y., T. Tashiro and H. Okubo (2000). Use of allozyme variation for evaluating genetic purity in asparagus (*Asparagus officinalis* L.) cultivars. Journal of Horticultural Science and Biotechnology, 75: 105-110.

Pharmawati, M., G. Yan and P. M. Finnegan (2005). Molecular variation and fingerprinting of Leucadendron cultivars (proteaceae) by ISSR markers. Ann.Bot. (Lond), 7: 1163-1170.

Popov, V. N., O. I. U. Urbanovich and V. V. Kiricenko (2002). Studying genetic diversity in inbred sunflower lines by RAPD and isoenzyme analysis. Genetika, 38: 937-943.

Sabu, K. K., P. Padmesh and S. Seeni (2001). Intraspecific variation in active principle content and isozymes of *Andrographis paniculata* Nees (Kalmegh): a traditional hepatoprotective medicinal herb of India. J. Medicinal and Aromatic Plant Science, 23: 637-647

Samy, A. A, Z. A. Kasem and A. S. Khaled (2007). Somaclonal variation in bread wheat (*Triticum asetivum* L.).4-RAPD: Fingerprinting of elite genotype under Siwa Oasis conditios. 8th African Crop Sci. Society Conf., El-Minia, Egypt, 1-13 Oct., 2007.

Täckholm, V. (1974). Student's Flora of Egypt, Ed: 2, Cairo, Cairo Univ.

Tso, S. C. and Y. R. Chen (1997): Isolation and characterization of a group III isozyme of acid phosphatase from rice plants. Bot. Bull. Acad. Sin., 38: 245-25.

Tsumura Y., K. Ohba and S. H. Strauss (1996). Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theor. Appl. Genet., 92: 40-45.

Welinder, K. G. (1992). Superfamily of plant, fungal and bacterial peroxidases. Curr. Opin. Struct. Biol., 2: 388-393.

Wendle, J. F. and N. F. Weeden (1989). Visualization and interpretation of plant isozymes. Isozymes in Plant Biology.Slotis, DE and Slotis P.S (Eds).

Zietkiewicz E., A. Rafalski and D. Labuda (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176-183.

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): Polymorphism percentages generated by five isozyme systems in the eleven *Brassicaceae* samples.

Type of isozyme	No. of mono-morphic bands	No. of poly-morphic bands	Total bands
α-Est	3	4	7
β-Est	3	4	7
Prx	2	3	5
Acph	2	4	6
Adh	2	0	2
Mdh	2	0	2
Total	14	15	29

Table (3): Nucleotide sequences and polymorphism of the ISSR primers among *Brassicaceae* species.

Primer Code No.	Primer Sequences	No. of monomor phic bands	No. of polymorp hic bands	Unique bands	Total bands	% polymorph ism
A17898	$(CA)_6$ AC	3	9	1	12	75.0%
B17898	$(CA)_6$ GG	1	6	1	7	85.7%
A17899	$(CA)_6$ AG	8	4	1	12	33.3%
B17899	$(CT)_8$ TG	5	5	1	10	50.0%
HB10	$(GA)_6$ CC	6	2	0	8	25.0%
HB12	$(GT)_8$ CC	4	5	0	9	55.6%
HB15	$(GTG)_3$ GC	5	4	1	9	44.4%
IS-O1	TTT $(TCC)_5$	4	2	1	6	33.3%
IS-O2	TTT $(TCC)_5$	3	4	0	7	57.1%
IS-O4	CAT $(CA)_7$ T	3	6	0	9	66.6%
IS-O5	ATTA $(CA)_7$	2	7	0	9	77.8%
IS-O6	$(GA)_8$ CG	4	6	1	10	60.0%
IS-O15	$(AG)_8$ CT	3	5	1	8	62.5%
IS-O12	$(TCC)_5$AC	1	6	0	7	85.7%
Total		52	71	8	123	57.7%

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

Primer Code No.	Plant species	Unique bands
A17898	*Brassica nigra*	1
B17898	*Anastatica heronitica*	1
A17899	*Eruca sativa* – Burg EL Arab	1
B17899	*Diplotaxis acris*	1
HB15	*Capsella brusa*	1
IS-O1	*Matthiola inaca*	1
IS-O6	*Diplotaxis harra*	1
IS-O15	*Brassica nigra*	1
Total		8

Table (5): Similarity matrix among the eleven *Brassicaceae* samples based on isozyme marker.

	1	2	3	4	5	6	7	8	9	10
2	.986									
3	.489	.489								
4	.486	.489	.989							
5	.405	.405	.405	.529						
6	.143	.143	.340	.340	.340					
7	.207	.207	.207	.193	.193	.193				
8	.340	.340	.397	.397	.397	.397	.633			
9	.095	.095	.193	.193	.193	.857	.853	.633		
10	.193	.193	.246	.246	.246	.486	.633	.849	.633	
11	.095	.095	.286	.286	.286	.930	.930	.714	.930	.571

Table (6): Similarity matrix among the eleven *Brassicaceae* samples based on ISSR.

	1	2	3	4	5	6	7	8	9	10
2	.974									
3	.970	.972								
4	.974	.974	.999							
5	.970	.978	.980	.998						
6	.513	.513	.503	.563	.591					
7	.550	.575	.563	.563	.998	.984				
8	.585	.566	.573	.573	.516	.979	.976			
9	.550	.550	.560	.560	.573	.989	.996	.979		
10	.504	.589	.589	.560	.560	.973	.979	.998	.979	
11	.506	.506	.573	.505	.505	.979	.987	.979	.998	.979

Table (7): Similarity matrix among the *Brassicaceae* samples based on ISSR and isozyme.

	1	2	3	4	5	6	7	8	9	10
2	.986									
3	.489	.489								
4	.486	.489	.989							
5	.405	.405	.405	.529						
6	.143	.143	.340	.340	.340					
7	.207	.207	.207	.193	.193	.193				
8	.340	.340	.397	.397	.397	.397	.633			
9	.095	.095	.193	.193	.193	.857	.853	.633		
10	.193	.193	.246	.246	.246	.486	.633	.849	.633	
11	.095	.095	.286	.286	.286	.930	.930	.714	.930	.571

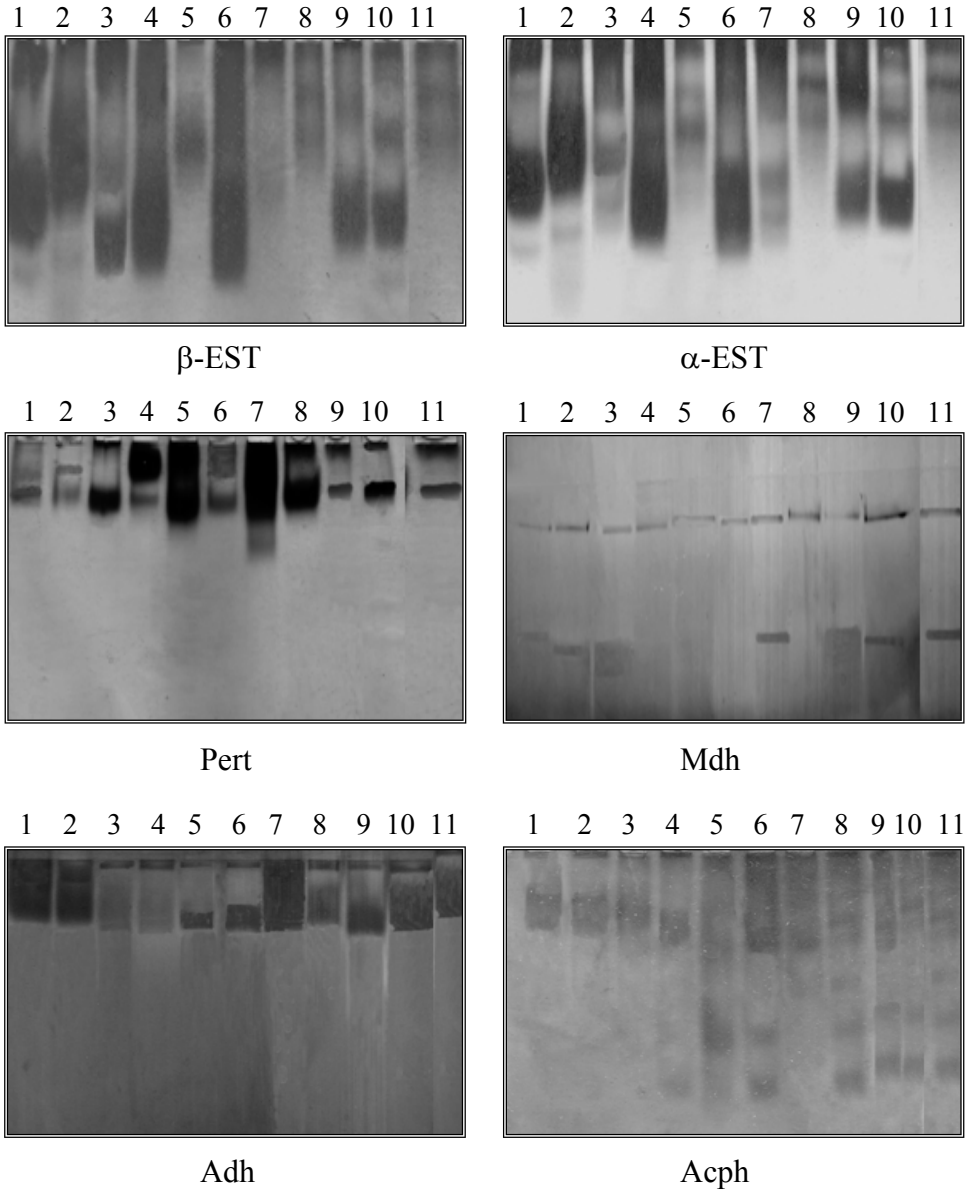

Fig. (1): Zymogram of β-Est, α-Est, Perx, Mdh, Adh and Acph banding patterns among the eleven *Brassicaceae* samples.

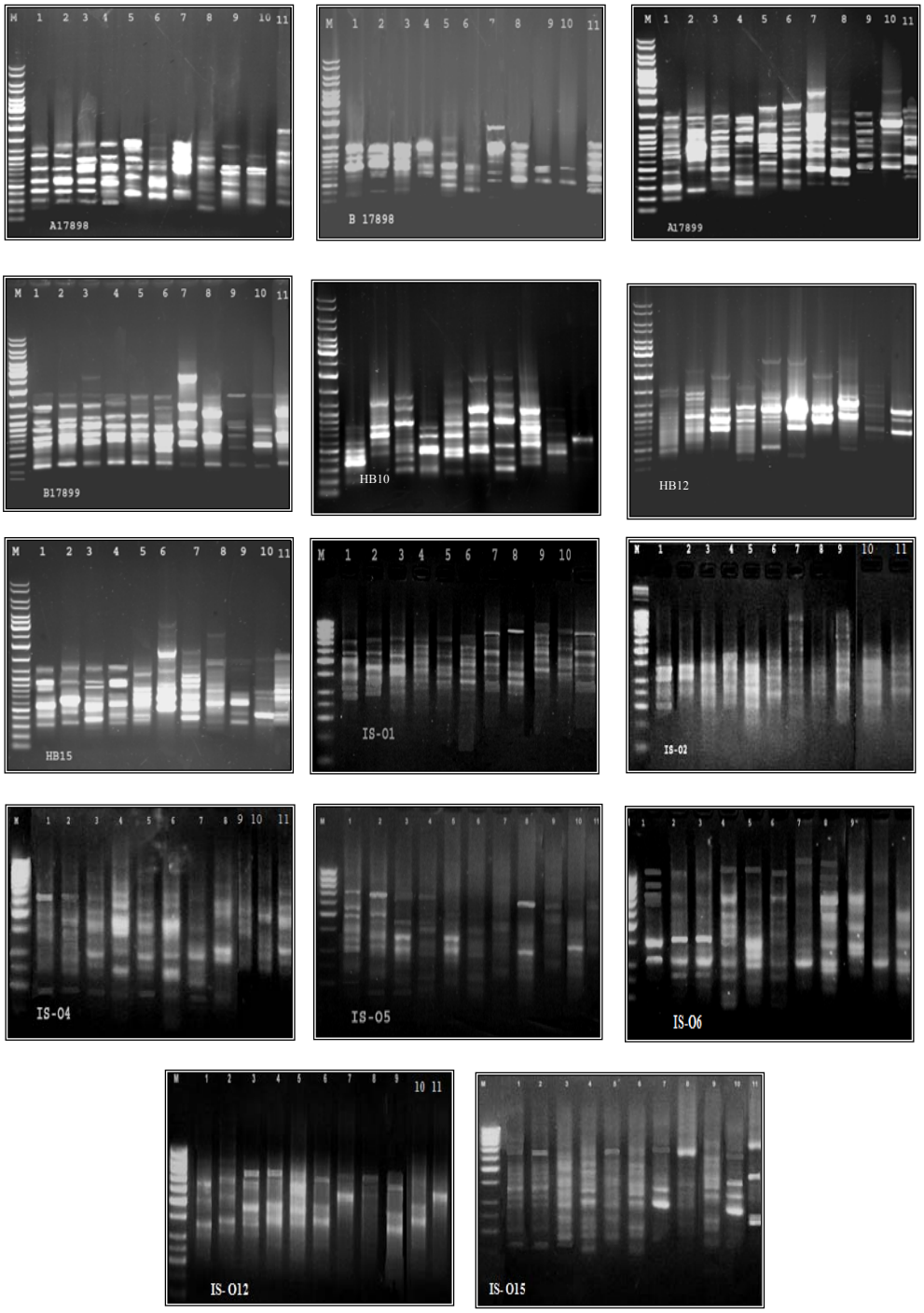

Fig (2): DNA polymorphism using ISSR with primers A17898, B17898, A17899, B17899, HB10, HB12, HB15, IS-O1, IS-O2, IS-O4, IS-O5, IS-O6, IS-O12 and IS-O15 in the 11 taxa of *Brassicaceae.*

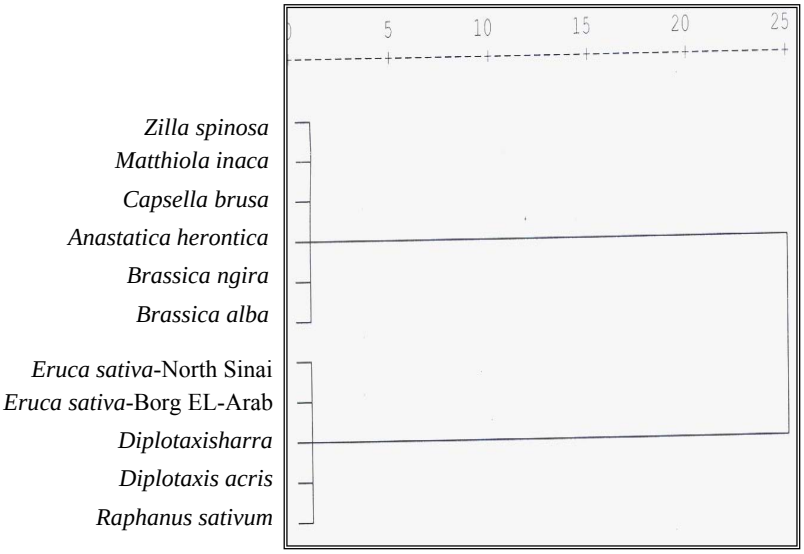

Fig. (3): Dendrogram based on isozyme of *Brassicacceae* species.

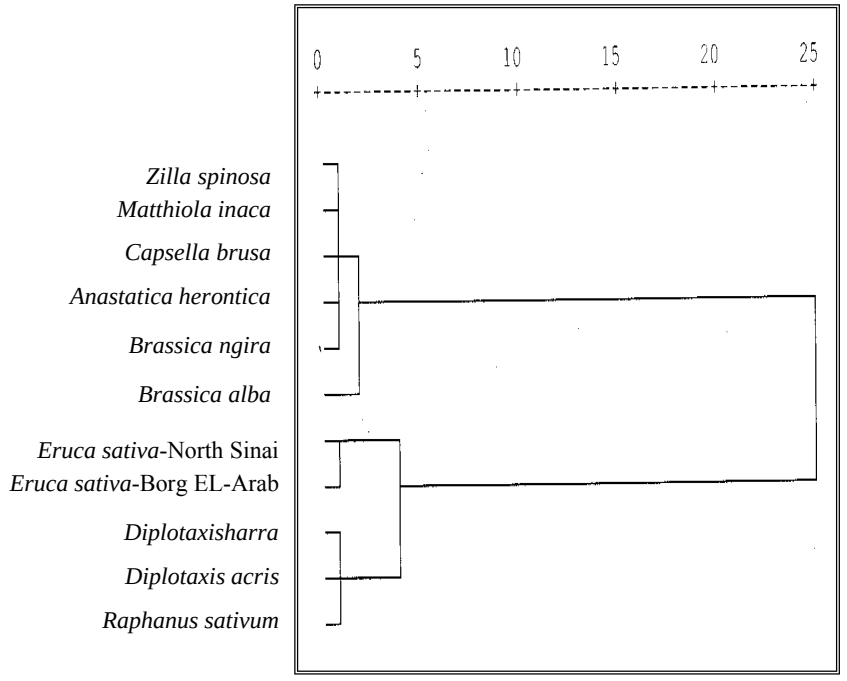

Fig. (4): Dendrogram based on ISSR of *Brassicacceae* species.

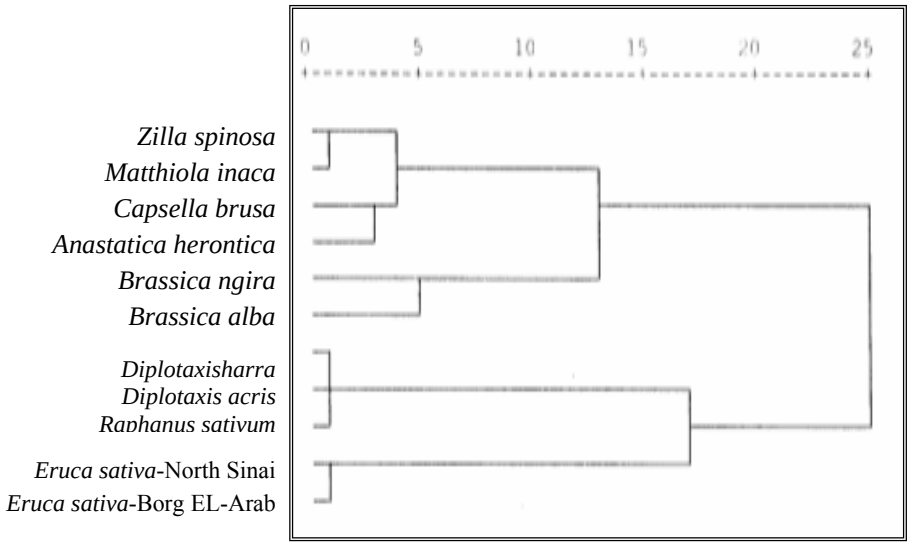

Fig. (5): Dendrogram based on ISSR and isozyme of *Brassicacceae* species.