ORIGINAL ARTICLE JGEB Vol. 6, No. 1, 2008 ISSN:1687-157X

Genetic Diversity Study in the Genus *Lathyrus* Using RAPD Analysis

Tahany A. El-Zoka[1] , Sawsan S. Youssef[2] and Hoda B. Mohamed[1]

[1]Genetics and Cytology Department, National Research Center, Dokki, Egypt, [2]Genetics Department,Faculty of Agriculture, Cairo University, Egypt.

ABSTRACT

Molecular markers based on PCR analysis using arbitrary 10-mer primers (RAPD) was used to identify and assess the genetic diversity of accessions of seven *Lathyrus* species: *L. aphaca, L. clymenum, L. ochrus, L. annuus, L. cicera* and *L. sativa tingitanus*. A total of 253 amplified products ranging in size from 300 bp to 3100 bp were generated by PCR amplification using 18 primers. Genetic similarities were estimated on the basis of pair-wise comparisons of RAPD marker analysis and dendrogram of genetic relationship between species were constructed. The RAPD data obtained were sufficient to distinguish between accessions and to separate these accessions by clustering them according to species. A high degree of genetic variability was recorded between studied species.

Key Words: *Lathyrus*, genetic diversity, RAPD.

Corresponding Author: T. A. Elzoka
E-mail : Tahanyelzoka@yahoo.com

Journal of Genetic Engineering and Biotechnology, 2008, 6(1): 11-19

INTRODUCTION

The analysis of genetic diversity and relatedness between or within different species, populations and individuals is a central task for many disciplines of biological science. With DNA being the only basis of genetic differences between distinct organisms and is much stable than proteins or enzymes, DNA fingerprinting is presently the ultimate method of biological individualization. As polymerase chain reaction (PCR) was developed *(Williams et al., 1990)*, it becomes a powerful tool for DNA fingerprinting. A PCR-based strategy involving the use of arbitrary primers to amplify random genomic DNA fragments (i.e. RAPD technique) is nowadays very popular due to its ability to rapid and easily generate polymorphic markers using a very small amount of starting DNA *(Thormann et al., 1994)*. Furthermore, RAPD markers can be used to detect genetic variation at the intraspecific level between closely related cultivars *(Kresovich, et al., 1992, Williams and Clair, 1993 and Abo-Elwafa et al., 1995)*.

The genus *Lathyrus* is the largest member of the viciae tribe (family Fabaceae) and has an importance as traditional foodstuffs in many cultures worldwide *(Kenicer et al., 2005 and Narayan et al., 1991)*. The genus consists of about 160 annual and perennial species,most of which are diploid *(Alkin et al.,1986)*. The species are subdivided into 13 sections based on their morpholo-gical traits *(Kupicha, 1983)*. Members of *Lathyrus* include food and fodder crops, ornamentals, soil nitrifies and model organisms for genetic and ecological research.

Taking into account the importance of cultivating *Lathyrus* species in the Mediterranean basin, a good knowledge of their biology and some organization of their genetic diversity is necessary to preserve. The present work was undertaken to find out the phylogenetic relationship between seven *Lathyrus* species *(i.e. L. aphaca, L. clymenum, L.ochrus, L.annes, L. cicera, L. sativa and L. tingitanus)* using RAPD analysis.

MATERIALS AND METHODS

Plant Materials:

Seeds of seven *Lathyrus* species, representing three sections in the genus *Lathyrus*, were chosen for examining interspecific variation. For intraspecific variation, four accessions from all species except *L. annuus* and *L. aphaca* where only two accessions were used (Table 1). Two plants from each accession were used as replicates to insure the results.

All accessions were kindly obtained from the germplasm collection of the Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.

Method:

Total genomic DNA was isolated from fresh young leaves (100 mg) of six week-old plants by Qiagen Kits using the PCR procedure reported by *Croft et al. (1999)*. Amplification reactions were performed in 25 µl volumes, containing PCR buffer (including 3 mM MgCl2), 1.0 U Taq DNA polymerase (Boehringer Mannheim Germany), 0.2 µM primer, 0.24 mM each of NTPs and 40 ng template DNA. PCR reactions were performed in a thermal cycler (Perkin Elmer-9600) programmed as follows: Initial denaturation at 94℃ for one minute, then 35 cycles of 94℃ (10 sec), 40℃ (30 sec), 72℃ (1 min) followed by a final extension at 72℃ (5 min). Amplified products from the RAPD reactions were separated by electrophoresis in 1.6 % agarose gel (Gibco BRL) in TAE buffer (sigma), stained with Ethidium Bromide (Roth). The reproducibility of the DNA profiles was tested by repeating the PCR amplification twice with each of the primers analysed. The obtained RAPD patterns were visualized by UV-light.

Primers which produced distinct, reproducible banding patterns were chosen for further analysis. Amplified products on each gel were scored as present (1) or absent(0) and pairwise comparisons between individuals were used to calculate Jaccard 's coefficient of genetic similarity matrix using Multi Variant Statistical Package (MVSP 3.1) computer program. Cluster analysis to produce a dendrogram was performed using unweighted pair-group method with arithmetical averages (UPGMA).

RESULTS

Eighteen primers were used to screen.0. the 24 accessions of seven different *Lathyrus* species.The frequent RAPD profiles of the 18 primers are shown in Figures from 1 to 5. The eighteen primers amplified a high number of polymorphic DNA products (253 markers), all of them were used to construct the dendrogram using Jaccard's coefficients (Figures 6 and 7). The total number of the amplified RAPDs produced by each primer varied from a minimum number of 8 amplified products by MPA-03 to a maximum of twenty amplified products by MP-10 (Table 3). The level of similarities between the different accessions is shown in (Table 4), while the level of similarities between the seven *Lathyrus* species studied is shown in Table 5.

The intra- and interspecific relationships were obtained by UPGMA analysis using Jaccard's coefficient. On the level of intra specific relationships between the different accessions *Lathyrus* within different species of *Lathyrus* (Figure 6 and Table 4), *L.ccicera* acessions showed the highest percentage of similarity in which accessions 1 and 2 were totally similar (100 %) as well as accessions 3 and 4. While *L. tingitanus* accessions showed the lowest level of variation, where the similarity between accessions 1 and 2 was 99 % and the same percentage was found between accessions 3 and 4, then comes *L. sativus*, in which the percentage of similarity between accessions 1 and 2 was 96 % and between accessions 3 and 4 was 97 %.

In *L. ochrus*, accessions 1 and 2 showed a percentage of similarity 94 % then comes accession 4 with 88% and accession 3 with 82%. On the other hand, *L.clymenum*, *L. aphaca* and *L.annuus* revealed high level of variation, which was very obvious to the two accessions of *L. aphaca* (the percentage of similarity was 52 %), then the accessions of *L. annuus* (the percentage of similarity was 70% and finally the accessions of *L. clymenum*, where the percentage of similarity between accessions 3 and 4 was 76 %, then comes accession 1 which was closer to accession 3 with 66 % and finally comes accession 2 which was closer to accession 4 with 47 %.

Table 1: *Lathyrus* species used for the examination of phylogenetic relationships.

Species	Accessions No.			
	1	2	3	4
L. ohrus	LAT 321/92	LAT 317/98	LAT 314/93	LAT 310/92
L. clymenum	LAT 112/92	LAT 130/93	LAT 147/95	LAT 165/96
L. aphaca	LAT 162/91	LAT 103/98		
L. annuus	LAT 145/96	LAT 152/87		
L. cicera	LAT 205/93	LAT 255/95	LAT 256/95	LAT 249/98
L. sativus	LAT 4033/91	LAT 4016/95	LAT 4017/95	LAT 487/98
L. tingitanus	LAT 108/98	LAT 109/96	LAT 106/79	LAT 140/70

Table 2: The sequence and GC% of the primers used for RAPD analysis.

Primer	Sequence 5`à3`	% GC	Primer	Sequence 5`à3`	% GC
MA-01	CAG GCC CTT C	70	MP-09	GTG GTC CGC A	70
MA-02	TGC CGA GCT G	70	MP-10	TCC CGC CTA C	70
MA-03	AGT CAG CCA C	60	MP-14	CCA GCC GAA C	70
MA-04	AAT CGG GCT G	60	MT-08	AAC GGC GAC A	60
MC-19	GTT GCC AGC C	60	MV-14	AGA TCC CGC C	70
MC-20	ACT TCG CCA C	70	MW-04	CAG AAG CGG A	60
MD-03	GTC GCC GTC A	60	MX-11	GGA GCC TCA G	70
MN-04	GAC CGA CCC A	70	MX-13	ACG GGA GCA A	60
MN-06	GAG ACG CAC A	60	MX-17	GAC ACG GAC C	70

Table 3: The total number of amplified products of the examined *Lathyrus* species scored per primer.

Primer	Number of amplified products							
	Total No. of amplified RAPDs	*L.ochrus*	*L.clymenum*	*L.aphaca*	*L.annus*	*L.cicera*	*L.sativus*	*L.tingitanus*
MA-01	15	7	6	3	7	5	5	5
MA-02	19	7	6	7	9	6	8	4
MA-03	8	3	4	4	3	4	2	4
MA-04	14	8	8	5	5	5	8	4
MC-19	9	7	5	3	4	3	3	4
MC-20	14	6	5	5	4	4	2	5
MD-03	14	7	8	3	7	4	3	4
MN-04	18	6	9	9	7	4	10	9
MN-06	12	6	4	3	2	5	4	6
MP-09	14	4	1	4	6	5	6	6
MP-10	20	5	10	6	6	8	6	6
MP-14	15	8	11	7	5	6	8	6
MT-08	16	5	5	2	3	3	5	2
MV-14	15	6	4	6	7	5	3	2
MW-04	15	7	10	10	6	2	4	6
MX-11	12	4	6	3	4	6	7	5
MX-13	11	7	6	4	5	5	5	6
MX-17	12	4	6	2	5	6	6	3
Total	253	107	106	86	95	86	95	87

On the interspecific level, the seven species were divided into two groups. In the first one, *L. ochrus* was related to *L. clymenum* and *L. aphaca* was related to *L. annuus*.

In the second group, *L. cicera* was close to *L. sativus*, then comes *L. tingitanus*. In this case the relationships between the species could take the following regular order: *L. ochrus, L. clymenum, L. aphaca, L.annuus, L. cicera, L. sativus* and *L. tingitanus* (Figure 7 and Table 5).

Table 4: The level of similarities between 24 accessions of *Lathyrus* species,produced by Jaccard's coefficient and expressed as percentage based on RAPD analysis.

L.ochrus 1	1.00																							
L.ochrus 2	0.94	1.00																						
L.ochrus 3	0.73	0.78	1.00																					
L.ochrus 4	0.86	0.88	0.82	1.00																				
L.clymenum 1	0.32	0.32	0.30	0.31	1.00																			
L.clymenum 2	0.40	0.39	0.36	0.41	0.45	1.00																		
L.clymenum 3	0.36	0.36	0.36	0.36	0.41	0.45	1.00																	
L.clymenum 4	0.37	0.36	0.33	0.35	0.63	0.47	0.76	1.00																
L.aphaca	0.17	0.18	0.19	0.19	0.22	0.18	0.18	0.20	1.00															
L.aphaca 2	0.26	0.27	0.25	0.25	0.22	0.23	0.21	0.25	0.52	1.00														
L.annuus 1	0.20	0.21	0.20	0.20	0.21	0.17	0.22	0.23	0.25	0.27	1.00													
L.annuus 2	0.16	0.17	0.18	0.17	0.21	0.18	0.21	0.22	0.23	0.24	0.70	1.00												
Lcicera 1	0.17	0.17	0.23	0.19	0.18	0.19	0.17	0.16	0.18	0.15	0.24	0.22	1.00											
Lcicera 2	0.17	0.17	0.23	0.19	0.18	0.19	0.17	0.16	0.18	0.15	0.24	0.22	1.00	1.00										
Lcicera 3	0.17	0.17	0.23	0.19	0.18	0.19	0.17	0.16	0.18	0.15	0.24	0.22	0.98	0.98	1.00									
Lcicera 4	0.17	0.17	0.23	0.19	0.18	0.19	0.17	0.16	0.18	0.15	0.24	0.22	0.98	0.98	1.00	1.00								
L.sativus 1	0.19	0.19	0.24	0.21	0.24	0.22	0.19	0.21	0.19	0.19	0.19	0.21	0.30	0.30	0.29	0.29	1.00							
L.sativus 2	0.18	0.18	0.24	0.20	0.23	0.20	0.19	0.20	0.19	0.18	0.20	0.22	0.31	0.31	0.30	0.30	0.96	1.00						
L.sativus 3	0.18	0.18	0.24	0.20	0.23	0.21	0.19	0.21	0.19	0.18	0.20	0.20	0.32	0.32	0.31	0.31	0.95	0.95	1.00					
L.sativus 4	0.18	0.18	0.24	0.20	0.23	0.20	0.19	0.20	0.19	0.18	0.21	0.20	0.32	0.32	0.31	0.31	0.94	0.96	0.97	1.00				
L.tingitanus 1	0.21	0.21	0.26	0.23	0.22	0.19	0.20	0.20	0.18	0.21	0.19	0.18	0.20	0.20	0.21	0.21	0.24	0.23	0.23	0.23	1.00			
L.tingitanus 2	0.20	0.20	0.25	0.22	0.22	0.18	0.19	0.19	0.18	0.20	0.20	0.18	0.20	0.20	0.21	0.21	0.24	0.23	0.23	0.23	0.99	1.00		
L.tingitanus 3	0.22	0.23	0.28	0.25	0.22	0.19	0.19	0.20	0.19	0.21	0.20	0.18	0.22	0.22	0.22	0.22	0.23	0.23	0.22	0.22	0.92	0.93	1.00	
L.tingitanus 4	0.22	0.23	0.28	0.25	0.22	0.19	0.19	0.20	0.19	0.21	0.20	0.18	0.21	0.21	0.22	0.22	0.24	0.24	0.23	0.23	0.93	0.94	0.99	1.00
	ochrus 1	ochrus 2	ochrus 3	ochrus 4	clyme num	clyme num	clyme num	clyme num	aphaca 1	aphaca 2	annuus 1	annuus 2	cicera 1	cicera 2	cicera 3	cicera 4	sativus 1	sativus 2	sativus 3	sativus 4	tigitanus 1	tigitanus 2	tigitanus 3	tigitanus 4

Table 5: The level of similarities between 7 Lathyrus species, produced by Jaccard's coefficient and expressed as percentage based on RAPD analysis.

L. ochrus	1.00						
L. clymenum	0.37	1.00					
L. aphaca	0.26	0.25	1.00				
L. annuus	0.20	0.23	0.27	1.00			
L. cicira	0.17	0.17	0.15	0.24	1.00		
L. sativus	0.19	0.21	0.19	0.19	0.29	1.00	
L. tinitanus	0.22	0.20	0.21	0.20	0.22	0.24	1.00

L. ochrus L.clymenum L.aphaca L. annuus L. cicera L. sativus L. Tingitanus

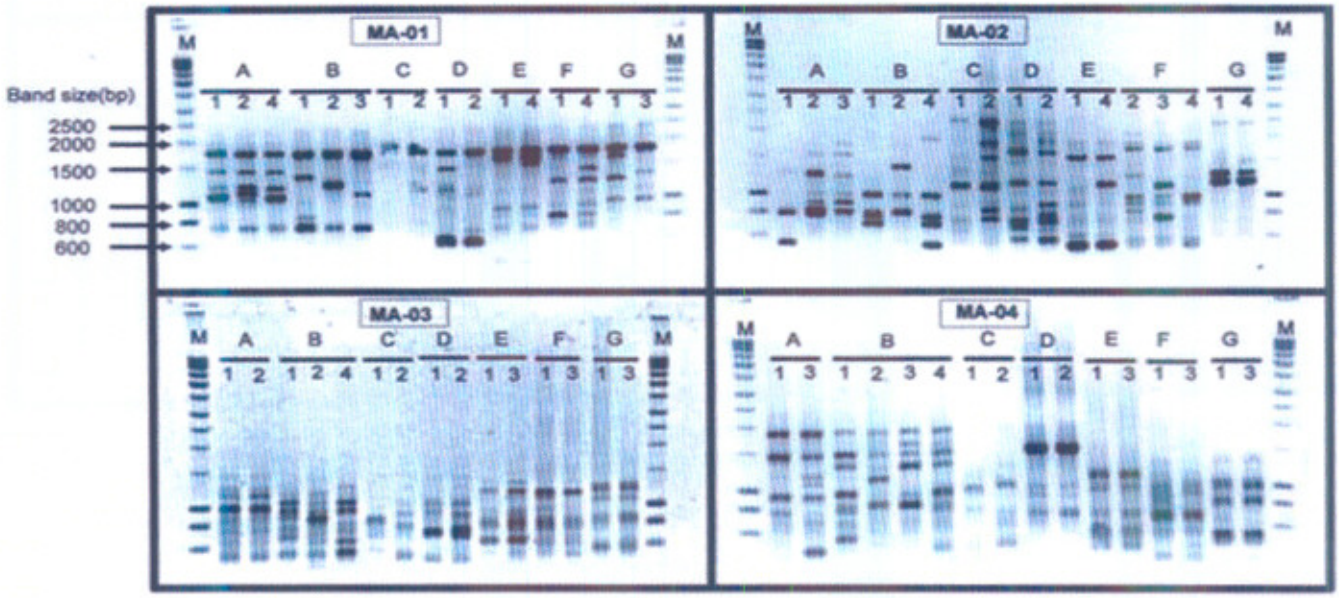

Figure 1: The frequent RAPD patterns in some of the different accessions (1-4) of the seven Lathyrus species . A) *L.ochrus* B) *L.clymenum* C) *L.aphaca* D) *L.annuus* E) *L.cicera* F) *L.sativus* G) *L.tingitanus* generated by four primers (MA-O1, MA-O2, MA-O3 and MA-O4) . M: molecular marker (Eurogenetic, 400 bp – 8 kb) .

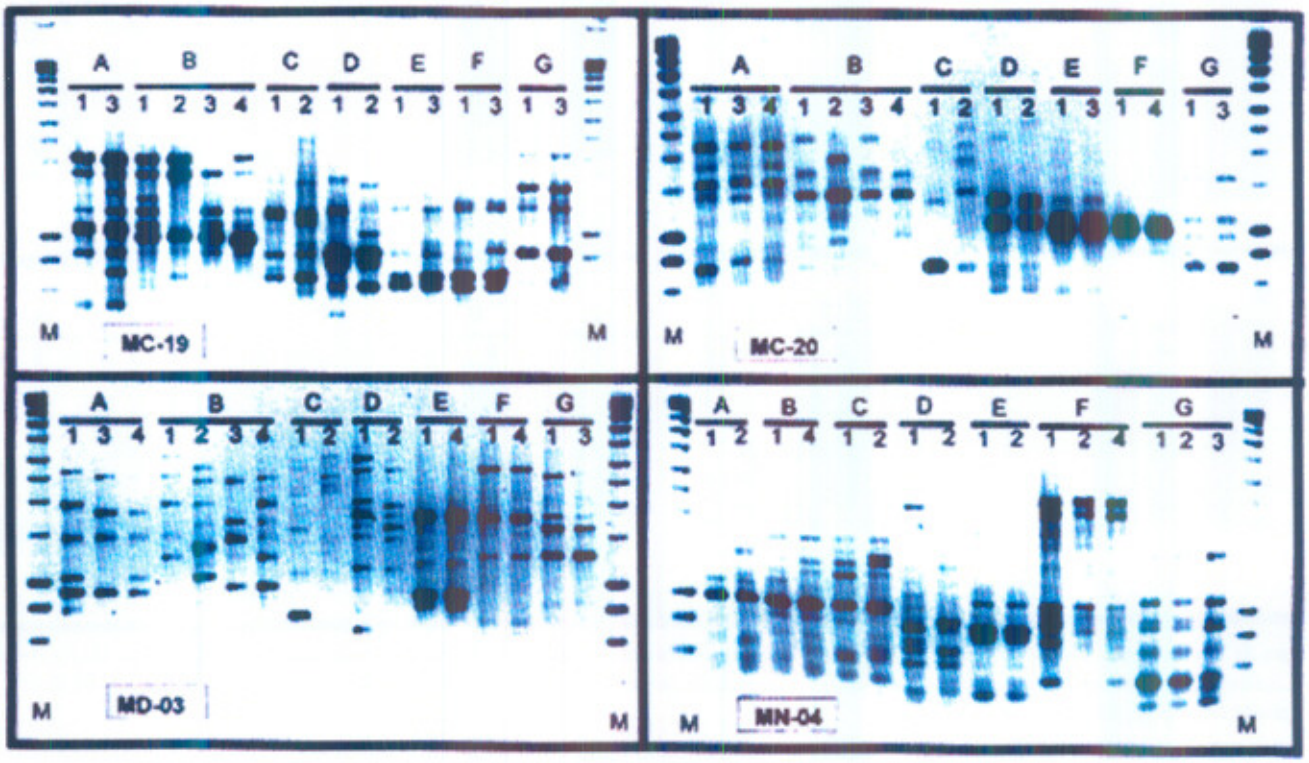

Figure 2: The frequent RAPD patterns in some of the different accessions (1-4) of the seven Lathyrus species .A) *L.ochrus* B) *L.clymenum* C) *L. aphaca* D) *L. annuus* E) *L.cicera* F) *L. sativus* G) *L. tingitanus* generated by four primers (MC -19 , MC-20 ,MD-03 , and MN-04) M : molecular marker (Eurogenetic, 400 bp-8 kb) .

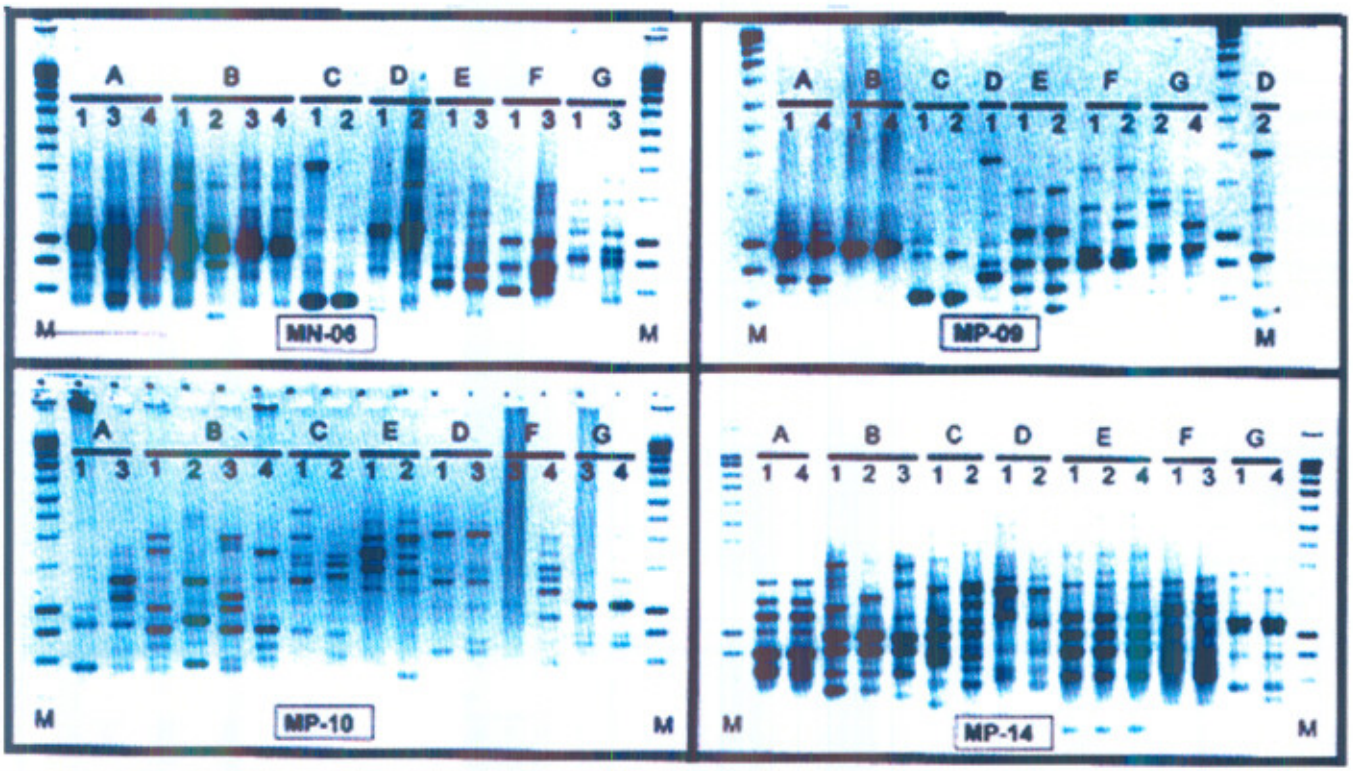

Figure 3: The frequent RAPD patterns in some of the different accessions (1-4) of the seven Lathyrus species. A) *L. ochrus* B) *L. clymenum* C) *L. aphaca* D) *L. annuus* E) *L. cicera* F) *L. sativus* G) *L. tingitanus* generated by four primers (MN-06 , MP-09 , MP-10 , and MP-14). M : molecular marker (Eurogenetic, 400 bp – 8 kb) .

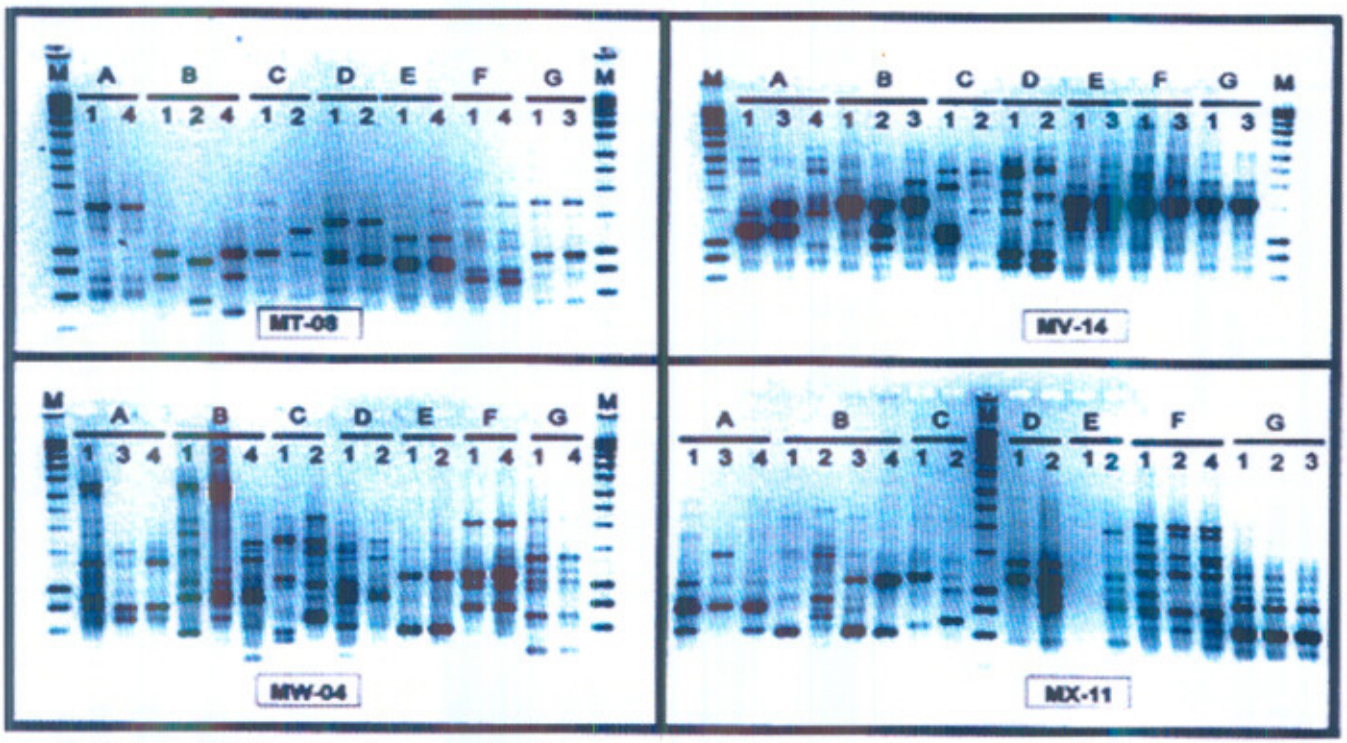

Figure 4: The frequent RAPD patterns in some of the different accessions (1-4) of the seven Lathyrus species . A) *L.ochrus* B) *L.clymenum* C) *L.aphaca* D) *L.annuus* E) *L.cicera* F) *L.sativus* G) *L.tingitanus* generated by four primers (MT-O8, MV-14, MW-O4 and M X-11) . M: molecular marker (Eurogenetic, 400 bp – 8 kb) .

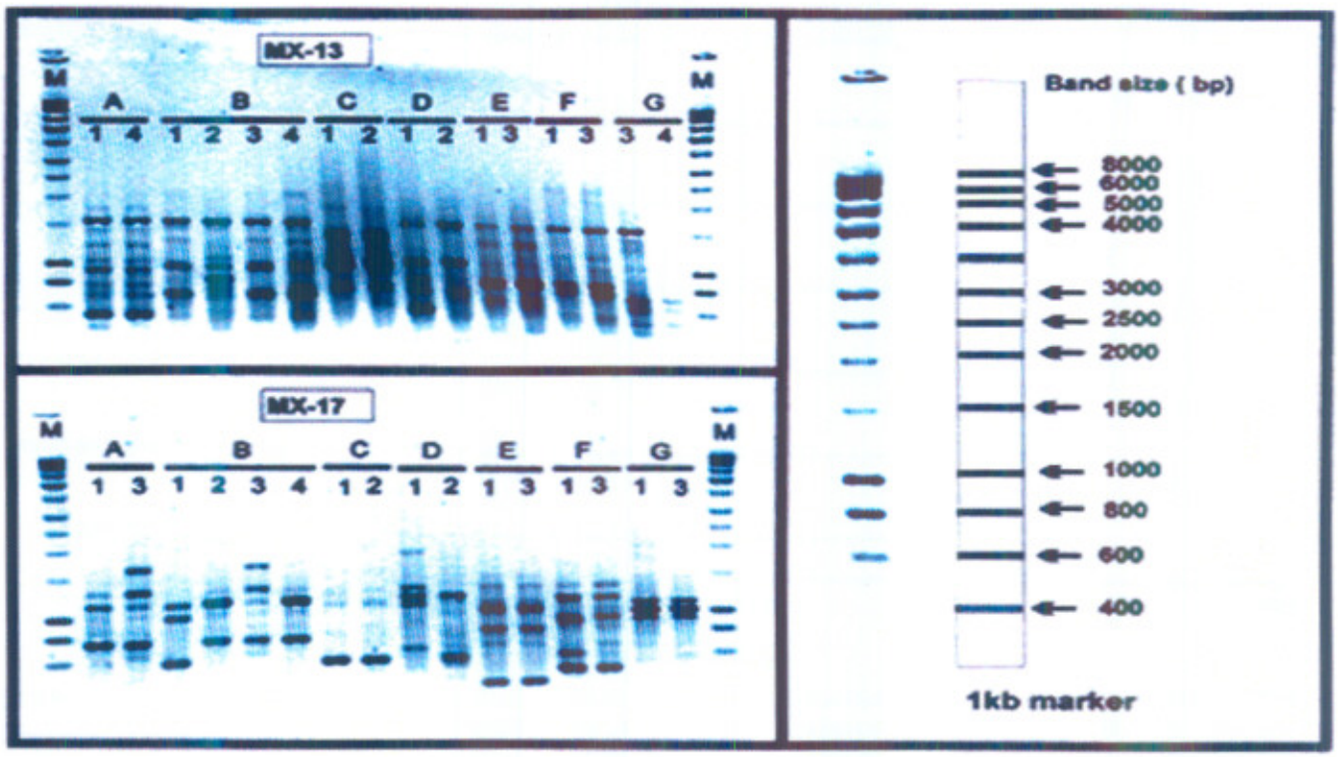

Figure 5: The frequent RAPD patterns in some of the different accessions (1-4) of the seven Lathyrus species . A) *L.ochrus* B) *L.clymenum* C) *L.aphaca* D) *L.annuus* E) *L.cicera* F) *L.sativus* G) *L.tingitanus* generated by two primers (MX-13 , MX-17) . M: molecular marker (Eurogenetic , 400 bp – 8 kb) .

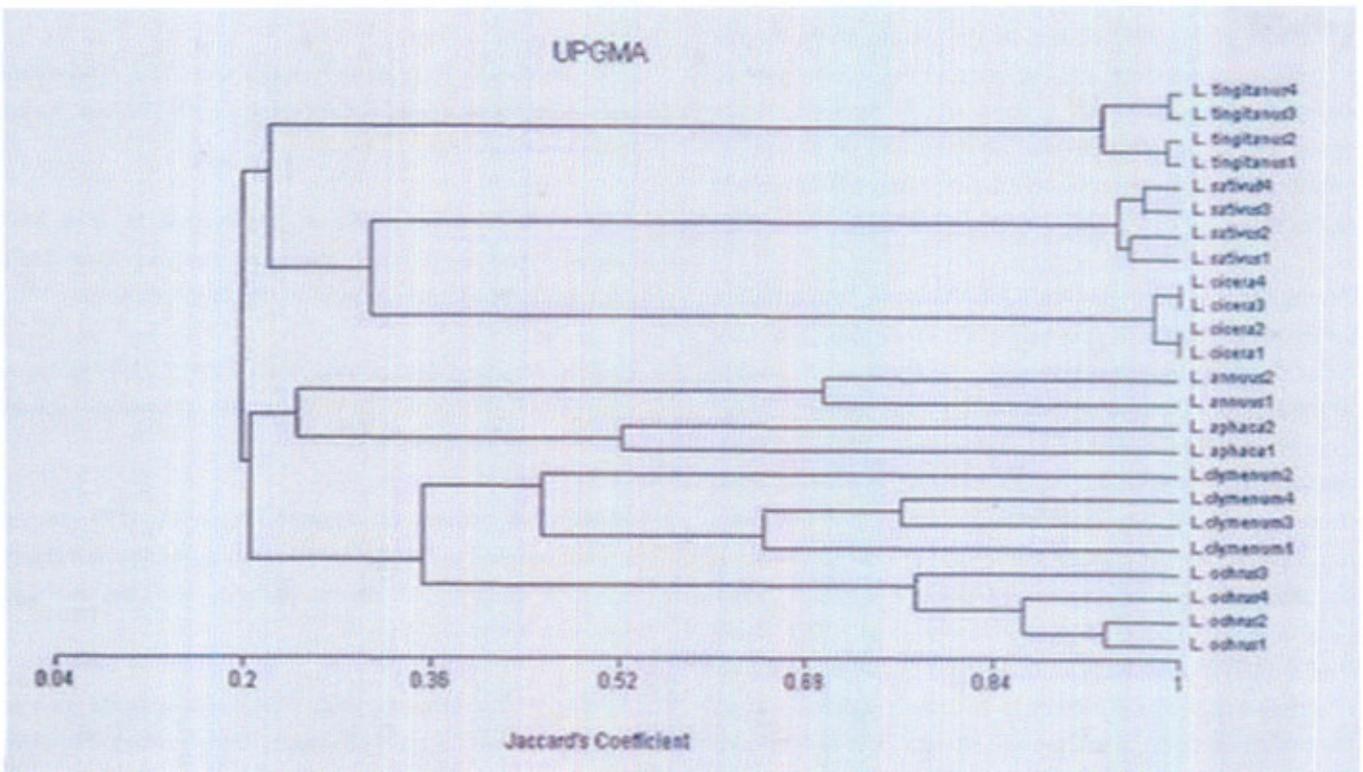

Figure 6: Dendrogram showing the genetic relationship between 24 accessions of seven *Lathyrus* species produced from pairwise comparisons of RAPD markers using Jaccard's coefficient of genetic similarities cluster analysis UPGMA methods .

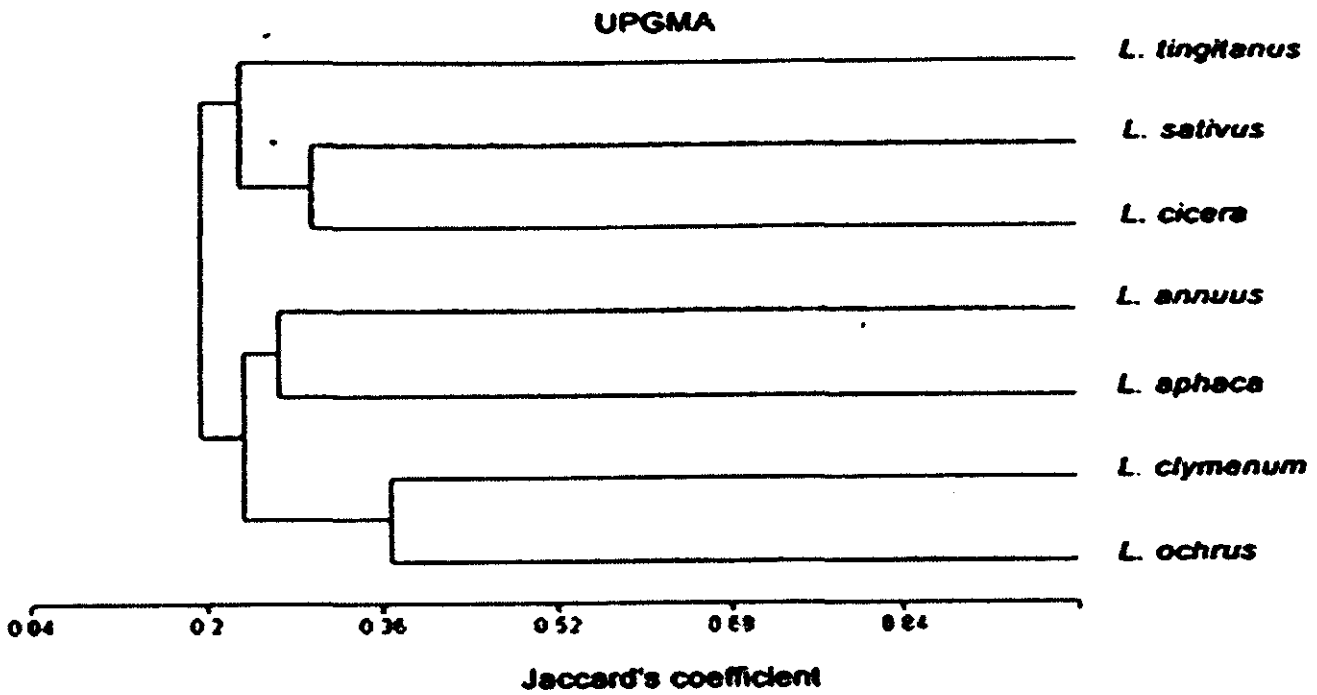

Figure 7: Dendrogram showing the genetic relationship between seven *Lathyrus* species produced from pairwise comparisons of RAPD markers, using Jaccard's coefficient of genetic similarity cluster analysis, using UPGMA methods.

DISCUSSION

The utility of PCR-based RAPD variations as phylogenetic markers for investigating evolutionary relationships among plants has been clearly established *(Ghislain et al., 1999 and Raina et al., 2001)*. Thus, in the present study, RAPD fingerprinting data were used to examine the level of genetic diversity within *Lathyrus* species and to assess to what extent the results of molecular analysis will be helpful in reconstructing phylogenetic relationships between species in this genus.

Polymorphisms obtained with RAPD markers have different under-lying causes at the molecular level and thus may differ for their informativeness in the analysis of genetic relationships. The results obtained in the present study support the classification of *L. ochrus and L. clymenum* into section Clymenum based on the morphological characters *(Kupicha, 1983)*. The results also confirm previous data based on morphological markers which have shown *L. cicera* are closely related to *L. sativus (Jackson and Yunus, 1984)*. *Croft et al. (1999)* and *Chtourou-Ghorbel et al. (2002)* using RAPD analysis found that for the species of section *Lathyrus*, *L. cicera* was the most similar to *L. sativus* which confirms the results obtained in the present study. These two species may be a result of hybridization or common ancestory. Some inter-specific crosses between the two have been successful *(Yunus and Jackson, 1991)*. A close phylogenetic proximity between *L. annuus* and *L. aphaca* was also shown in the present study.

REFERENCES

Abo-Elwafa, A., Murai, K. and Shimada, T. 1995. Intra- and inter-specific variations in Lens revealed by RAPD markers. Theoretical and Applied Genetics **90**(3 -4) :335--340.

Allkin, R., Goydir, D. J., Bisby, F. A. and white, R. J. 1986. Names and synonyms of species and subspecies in the Vicieae. Vicieae Data Base Project **7**(3): 1 - 75.

Chtourou-Ghorbel, N., Lauga, B., Ben Brahim, N., et al. 2002. Genetic variation analysis in the genus Lathyrus using RAPD markers. Genetic Resources and Crop Evolution **49**(4):363--370.

Croft, A. M., Pang, E. C. K. and Taylor, P. W. J. 1999. Molecular analysis of Lathyrus sativus L. (grasspea) and related Lathyrus species. Euphytica **107**(3):167--176.

Ghislain, M., Zhang, D., Fajardo, D., et al. 1999. Marker-assisted sampling of the cultivated Andean potato Solanum phureja collection using RAPD markers. Genetic Resources and Crop Evolution **46**(6):547--555.

Jackson, M. T. and Yunus, A. G. 1984. Variation in the grass pea (Lathyrus sativus L.) and wild species. Euphytica **33**(2):549--559.

Kenicer, G. J., Kajita, T., Pennington, R. T. and Murata, J. 2005. Systematics and biogeography of Lathyrus (Leguminosae) based on internal transcribed spacer and cpDNA sequence data. American Journal of Botany **92**(7):1199--1209.

Kresovich, S., Williams, J. G. K., McFerson, J. R., et al. 1992. Characterization of genetic identities and relationships of Brassica oleracea L. via a random amplified polymorphic DNA assay. Theoretical and Applied Genetics **85**(2 -3):190- 196.

Kupicha, F. K. 1983. The infrageneric structure of Lathyrus. Notes from the Royal Botanic Garden, Edinburgh **41**(2):209- 244.

Narayan, R. K. J. 1991. Molecular organisation of the plant genome: Its relation to structure, recombination and evolution of chromosomes. Journal of Genetics **70**(1):43--61.

Raina, S. N., Rani, V., Kojima, T., et al. 2001. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (Arachis hypogaca) cultivars and wild species. Genome **44**(5):763--772.

Thormann, C. E., Ferreira, M. E., Camargo, L. E. A., et al. 1994. Comparison of RFLP and RAPD markers for estimating genetic relationships within and among cruciferous species. Theoretical and Applied Genetics **88**(8):973--980.

Williams, C. E. and St. Clair, D. A. 1993. Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of Lycopersicon esculentum. Genome **36**(3):619--630.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., et al. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research **18**(22):6531--6535.

Yunus, A. F. and Jackson, M. T. 1991. The gene pools of the grasspea (Lathyrus sativus L.). Plant Breeding **106**(4):319--328.