Genetic diversity of wild and common bean (*Phaseolus spp.)* genotypes as revealed by RAPD And AFLP markers

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ABSTRACT

The present work was conducted to evaluate RAPD-PCR and AFLP (amplified fragment length polymorphism) marker systems for their ability to detect genetic diversity within and among some common bean (Phaseolus vulgaris)cultivars and tepary bean (Phaseolus acutifolius) lines and to compare the efficiency of these two marker types in the classification of accessions according to the gene pool of beans. The polymorphic fragments were obtained on the basis of 12 differentiating primers using the RAPD method and 4 differentiating primer combinations using the AFLP method. The 12 RAPD primers produced 119 polymorphic bands, while AFLP primer combinations produced 165 polymorphic bands. RAPD data analysis showed that the genetic similarity among thirteen Phaseolus accessions ranged from 44.6 to 93.8% while the AFLPs generated data show that the highest genetic similarity value was 86.7% and the lowest value was 27.7% with an average of 57.2%. The dendrogram generated with hierarchical UPGMA (unweighted pair group method with arithmetic mean) cluster analysis of the Jaccard's similarity coefficient matrices revealed two major clusters, which were identified.

Keywords: *AFLP, Genetic diversity, Phaseolus vulgaris, Phaseolus acutifolius, RAPD.*

INTRODUCTION

The genus *Phaseolus* originated in the Americas and contains 55 species, six of which are widely cultivated: *Phaseolus vulgaris* L., *Phaseolus lunatus* L., *Phaseolus coccineus* L., *Phaseolus acutifolius* A., *Phaseolus latifolius* and *Phaseolus polyanthus* (Debouck, 1999). Of these six species, the common bean (*Phaseolus vulgaris* L.) is the most important because its high protein content has made it the major staple food crop in Africa and Latin America (Yu and Bliss, 1978), although, tepary and common bean have the same number of chromosomes (2n=22) and similar karyotypes (Marechal, 1970).

Amplified fragment length polymorphism (AFLP) is a polymerase chain reaction (PCR)-based molecular marker assay (Vos *et al.*, 1995) that can detect a higher number of polymorphic loci in a single assay than restriction fragment length polymorphism (RFLP) or random amplification of polymorphic DNA (RAPD) (Powell *et al.*, 1996). AFLP is highly polymorphic and shows considerable reproducibility within a laboratory.

The effectiveness and reliability of AFLP has led to its increasing use in diversity studies, phylogeny, genomic linkage mapping and identification of varieties (Tohme *et al.*, 1996; Papa and Gepts, 2003; Rosales-Serna *et al.*, 2005). AFLPs can detect a large number of polymorphic bands in a single lane rather than high levels of polymorphism at each locus such as with SSRs. AFLP has been used to distinguish very closely related genotypes belonging to the same commercial class, such as the yellow bean class (Gaita´n-Solıs *et al*., 2002; Pallottini *et al*., 2004; Guerra-Sanz, 2004; Caixeta *et al*., 2005; Buso *et al*., 2006; Benchimol *et al*.,2007; Campos *et al*., 2007; Hanai *et al*.,2007 and Cardoso *et al*.,2008).

A comparison of the different marker systems used to estimate crop genetic diversity is important in order to assess their usefulness in germplasm conservation and as plant breeding tools. Garcia *et al*., (2004) used different marker systems (AFLP, RAPD, RFLP and SSR) to examine the diversity of tropical maize inbred lines and concluded that AFLP was the best molecular assay for fingerprinting and assessing genetic relationships because of its high accuracy.

Geleta *et al*. (2005) reported that both AFLP and SSR markers were efficient tools in assessing the genetic variability among sorghum genotypes.

In this study, we sought to determine the most suitable molecular marker system (RAPD or AFLP) for assessing the genetic diversity in commercial and wild beans and examine the genetic variability among various genotypes.

MATERIALS AND METHODS

1. Plant material and DNA extraction

A set of 11 common bean cultivars and two wild accessions (G40083 and G40084) of *Phaseolus acutifolius* (2n=22) were evaluated under drought stress. The 11 *Phaseolus vulgaris* genotypes included belonged to the gene pools of common bean (Polysta, Nebraska, Gomi, Ferrary, Mv309, Giza-9, Sonate, Goru, Argus, Xera and Amel). Seeds of these cultivars were obtained from the National Germplasm Resources Laboratory, Beltsville, USA and Germplasm Preservation Laboratory, Faculty of Agriculture at Moshtohor, Benha University and Horticulture Research Institute, Agricultural Research Center, Dokki, Egypt. Total genomic DNA was extracted from young leaf tissue using the CTAB extraction method as described by (Doyle and Doyle,1987).

Phylogenetic relationships and genetic diversity were assessed for these accessions and among other cultivated forms of *Phaseolus vulgaris* using data generated from RAPD and AFLP molecular marker techniques.

2. RAPD analysis

Oligonucleotide sequences of the 10-mer random primers used in this study were selected from a set of Operon kits (Operon Technologies Inc., Alameda California, USA). A total of twelve random 10-mer primers as indicated in Table (1) were used in the detection of polymorphism among *Phaseolus vulgaris* ssp. PCR reactions were conducted according to (Williams *et al.*, 1990 and Driessen *et al.*, 2001). The reaction conditions were optimized and 25 ml total volume mixtures were composed of dNTPs 200 mM, $MgCl_2$ 1.5 *m*M, 1X buffer, primer 0.2 *m*M, DNA 100 ng, and Taq DNA polymerase(Promega,USA) 2 units. Negative control was included where all the ingredients were present except template DNA. Amplification were carried out in a

thermocycler (UNO II, Biometra) programmed for 95°C for 5 min (one cycle); followed by 94°C for 1 min, 35°C for 1 min and 72°C for 2 min (45 cycles); 72°C for 5 min (one cycle), then 4°C (infinitive).

Amplification products (7μl) were mixed with 3 μl loading buffer and separated on 1.5% agarose gel and stained with 0.5 *mg*/ml ethidium bromide, and visualized with ultraviolet light and photographed. DNA fragment sizes were determined by comparisons with the 100 bp DNA ladder marker (Promega, USA).

Table (1): Sequence of twelve decamer arbitrary (10-mer) primers assayed in RAPD- PCR marker.

Primer	Sequence (5′ - 3′)	Primer	Sequence (5′ - 3′)
OPA-02	TGCCGAGCTG	**OPD-01**	ACCGCGAAGG
OPA-09	GGGTAACGCC	**OPD-02**	GGACCCAACC
OPB-05	TGCGCCCTTC	**OPD-10**	GGTCTACACC
OPB-07	GTCCACACGG	**OPD-16**	AGGGCGTAAG
OPC-06	GAACGGACTC	**OPE-07**	TCAGGGAGGT
OPC-20	ACTTCGCCAC	**OPG-03**	AGGCTGTGCT

3. AFLP analysis

AFLP analysis was performed using the Invitrogen AFLP® Analysis system I, and AFLP Starter primer kit (Cat # 10544-013 and 10483-014, respectively, Invitrogen Corporation, Carlsbad, California, USA) as indicated in Table (2). Amplicon- template preparation, pre-amplification and selective amplification were conducted according to manufacturer's protocol of the BRL AFLP analysis system I kit**(**Gibco,USA) for a small genome using a total of 250 ng of genomic DNA in *EcoRI*(E) and *MseI*(M) digestion.

Amplification products were electrophoresed in 6% (w/v) denaturing polyacrylamide sequencing gels for 2h at 2000 W, and DNA bands were visualized by silver staining according to methods of (Vos et *al*., 1995). All the polymorphic AFLP bands between 100 and 500 bp were scored and fragments were sized by comparison at a 100 bp ladder molecular weight size standard.

4. Data analysis

Amplified products for RAPD and AFLP markers were visually examined for each primer or each primer combination. Also, Gel Documentation System (Gel-Doc 2000, with Diversity Database Fingerprinting Software, version 2.1, Bio-Rad Laboratories, and Hercules, California, USA) was used for gel analysis, scoring, data handling, cluster analysis and construction of dendrograms.

The obtained data of RAPD and AFLP analyses was entered in a computer file as binary matrices where “0”and “1”stand for the absence and presence, of a band, respectively in each individual sample. Similarity coefficients were calculated according to dice matrix (Nei and Li, 1979 and Rohlf, 1993). Parents were grouped by cluster analysis with the similarity matrix and unweighted pair group method based on arithmetic mean (UPGMA).

Table (2): Sequence of different AFLP primers used.

Primer	Sequence (5` - 3`)
MseI forward adapter	GACGATGAGTCCTGAG
MseI reverse adapter	TACTCAGGACTCAT
EcoRI forward adapter	CTCGTAGACTGCGTACC
EcoRI reverse adapter	AATTGGTACGCAGTCTAC
MseI primer core region (M)	GATGAGTCCTGAGTAA
MseI pre-selective primer (M+1)	M + C
MseI selective primers (M+3)	
M-CAA	GATGAGTCCTGAGTAACAA
M-CAG	GATGAGTCCTGAGTAACAG
M-CTG	GATGAGTCCTGAGTAACTG
M-CTC	GATGAGTCCTGAGTAACTC
EcoRI primer core region (E)	GACTGCGTACCAATTC
EcoRI pre-selective primer (E+1)	E + A
EcoRI selective primers (E+3)	
E-AAG	GACTGCGTACCAATTCAAC
E-ACA	GACTGCGTACCAATTCACA
E-AAC	GACTGCGTACCAATTCACC
E-ACA	GACTGCGTACCAATTCACA

RESULTS AND DISCUSSION

1. Identification of RAPD markers

The twelve primers that generated reproducible and scorable polymorphic markers were selected for further analysis. They produced multiple band profiles with a number of amplified DNA fragments ranging from 9.0 to 19.0, while the number of polymorphic fragments ranged from 4.0 to 15.0 (Table 3 and Figure 1).

A maximum number of 19.0 fragments was amplified with primer OPE-07 and the minimum number of 9.0 fragments was amplified with primers OPD-01 and OPD-02 (Table 3).

The total number of reproducible fragments amplified by the twelve primers reached 166.0 bands, of which 119.0 were polymorphic fragments. This represented a level of polymorphism of 70.16%, which indicates a very high level of polymorphism among the genotypes studied. The size of the amplified fragments also varied with the different primers and ranged from 100 to 1500 bp as shown in Fig. (1).

In the present study, RAPD analysis revealed a high level of polymorphism among the various genotypes studied, which enabled accurate analysis of the genetic distance.

The obtained results are in good accordance with those of (Shen *et al.*, 1996; Thompson *et al.*, 1997; Shen *et al.*, 1998; Ruas *et al.*, 1999; Wang and Goldman, 1999; Driessen *et al.*, 2001 and Zitoun *et al*., 2008).

Table (3): Total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by RAPD markers among the 13 Phaseolus spp. genotypes.

Primers	Total number of bands	Monomorphic Bands	Polymorphic Bands	% Polymorphism
OPA-02	14	6	8	57.15
OPA-09	14	4	10	71.43
OPB-05	15	3	12	80.00
OPB-07	16	5	11	68.75
OPC-06	11	5	6	54.55
OPC-20	13	0	13	100.00
OPD-01	9	4	5	55.56
OPD-02	9	5	4	44.45
OPD-10	15	0	15	100.00
OPD-16	15	3	12	80.00
OPE-07	19	5	14	73.69
OPG-03	16	7	9	56.25
Total	166	47	119	70.16

1.2 Genetic similarity within and among *Phaseolus spp*. Genotypes

The genetic similarity within and among thirteen *Phaseolus* spp. was estimated in terms of using Dice's similarity coefficients (DSC's) to compute the similarity matrix based on the scored RAPD data matrix.

This similarity matrix was used to generate a dendrogram using the UPGMA method. RAPD data analysis showed that the genetic similarity among thirteen *Phaseolus acutifolius* and *Phaseolus vulgaris* genotypes ranged from 44.6 to 93.8% as shown in Fig. (2). In addition, the highest similarity level (93.8%) was detected between wild tepary bean accessions G40083 and G40084 which are closely related accessions. While, the lowest genetic similarity (44.6%) was detected between wild tepary bean accessions (G40084) and Giza-9 bean cultivar.

The dendrogram separated the thirteen *Phaseolus vulgaris* and *Phaseolus acutifolius* genotypes two major clusters, according to the geographic distributions of wild accessions and to traditional classification of cultivated forms of bean (Carmenza *et al.*, 2006, Munoz *et al.*, 2004 and Svetleva *et al.*, 2006). The first cluster contained wild bean accessions collected from Mexico and southwestern USA (G40083 and G40084), while the second cluster contained all cultivated forms of bean, that could be divided into two subclusters. Bean cultivars Nebraska, Gomi and Goru are grouped together in the first subcluster, while bean cultivars Polysta, Ferrary, Mv309, Giza-9, Sonate, Argus, Xera and Amel grouped together in the second subcluster. The obtained results are in harmony agreement with those obtained by (El-Fiky and Wafaa, 2002; Monaj *et al.*,2005 and Franklin *et al.*, 2009).

1.3 Unique markers and fingerprints revealed by RAPD

In RAPD analysis among the twelve RAPD primers used, only one RAPD primer (OPD-02) failed to generate unique markers while the other 11 primers were able to generate unique markers (unique positive and/or negative markers) that could be used to identify bean genotypes with the percent of 92.3%. One RAPD primer produced unique negative markers (OPD-10), while three primers produced, unique positive markers (OPA-02, OPA-09 and OPE-07) and eight RAPD primers produced both types of unique markers (positive and negative). A total of 38 unique markers were generated from eleven primers of which twenty-two markers were unique positive and sixteen markers were unique negative with molecular weight ranging from 70 to 1500 bp (Table 4).

Table (4): Unique positive* and/or negative** markers, marker size and total number of unique markers revealed from RAPD assayed primers.

Primers	Unique RAPD markers				Total
	UPM*	Size (*bp*)	UNM**	Size (*bp*)	
OPA-02	2	480,600	0	-	2
OPA-09	3	100, 190, 520	0	-	3
OPB-05	3	180,270,400	3	70,480,600	6
OPB-07	2	460,390	1	290	3
OPC-06	2	210,400	2	500,700	4
OPC-20	2	380,620	2	200,400	4
OPD-01	1	400	1	1500	2
OPD-10	0	-	1	280	1
OPD-16	4	150,180,300,410	3	100,500,700	7
OPE-07	1	700	0	-	1
OPG-03	2	450,600	3	100,150,300	5
Total		22		16	38

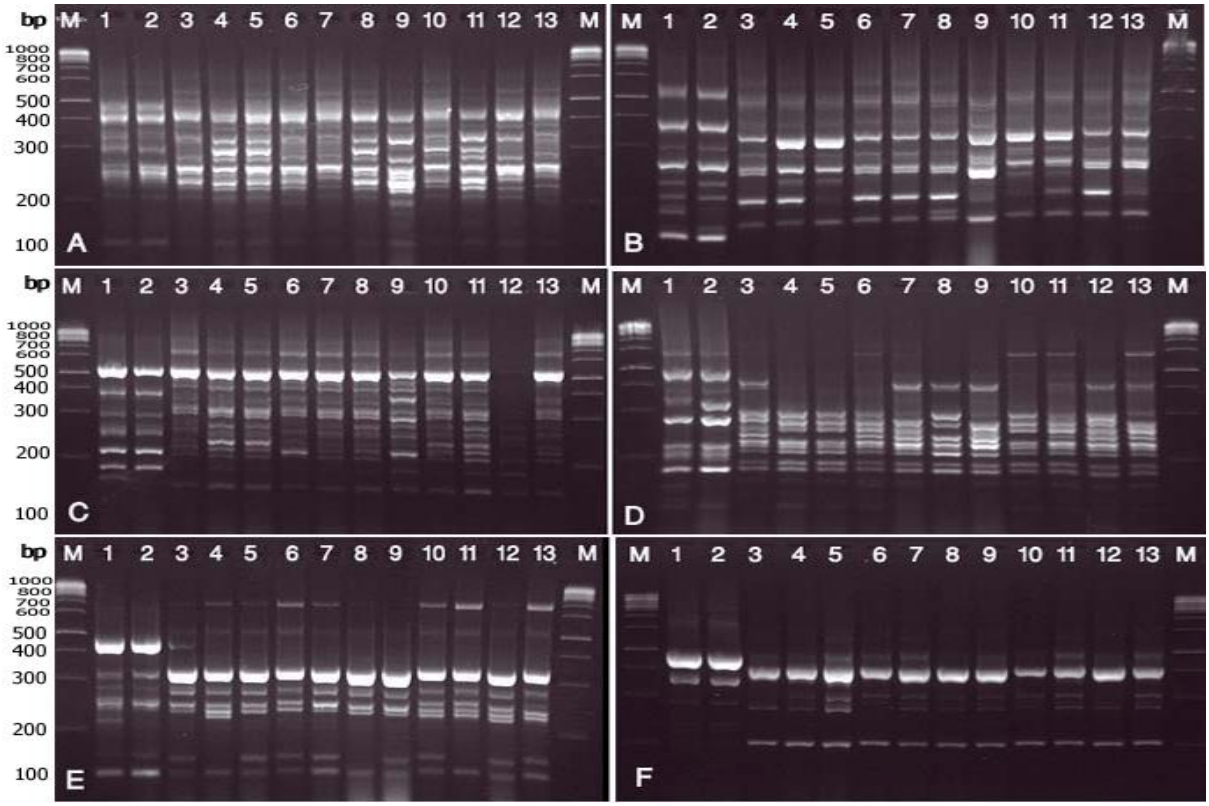

Fig. (1): RAPD profiles of 13 bean genotypes (1 - 13) as detected with primers (A) OPA-02, (B) OPA-09, (C) OPB-05, (D) OPB-07, (E) OPC-06 and (F) OPC-20. Lanes 1 and 2 represent wild bean accessions (G40083 and G40084) and lanes 3 to 13 represent: common bean relatives (Polysta, Nebraska, Gomi, Ferrary, Mv309, Giza-9, Sonate, Goru, Argus, Xera and Amel, respectively). DNA molecular weight standards of 100 bp DNA ladder.

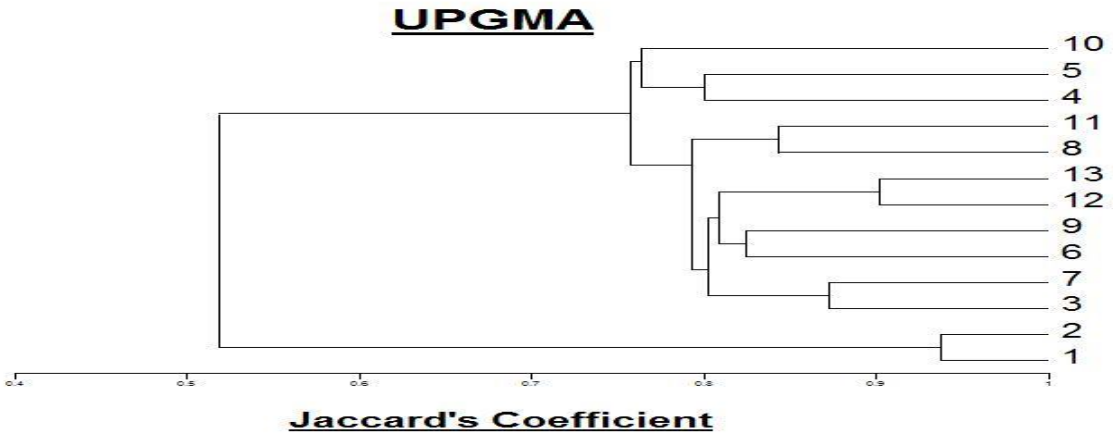

Fig. (2): Dendrogram for the 13 bean accessions constructed from the RAPDs generated data using UPGMA method and similarity matrices computed according to DSC's.

2.1 Identification of AFLP-PCR molecular markers

Four *EcoR1* and *Mse1* selective primer combinations were used for fragment amplification. All AFLP polymorphisms were scored as dominant marker. Sequences of selective primers used in assaying bean genotypes are given in Table (5) and Figure (3). AFLP primer combinations used generated 230 selectively amplified DNA fragments and identified 165 polymorphic markers as shown in (Table 5). On average 57.5 distinguishable bands were observed after selective amplification with each primer combination, and an average of 71.73 % of these AFLP bands were found to be polymorphic among bean genotypes, while, the percentage of polymorphism ranged from 67.21 to 76.92%. The highest percentage of polymorphism (76.92%) was obtained with primer combination, E-**ACA** X M-**CAG**, while the least percentage (67.21%) was obtained with the primer combination E-**AAC** X M-**CTC**.

However, the maximum number of bands per primer combination was found to be 69.0 which was generated with primer combination E-AAC X M-CTG, whereas the minimum number of bands per primer combination (48) was obtained with primer combination E-AAG X M-CAA, (Table 5 and Fig. 3), confirming the high multiplex ratio produced by AFLP markers. The size of selectively amplified DNA fragments ranged in size from 50 to 600 *bp* as shown in Fig. (3). These results are in good accordance with the results obtained by (Maras *et al.*,2008).

Table (5): Total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by AFLP markers among the 13 bean genotypes.

Primer combinations	Total number of bands	Monomorphic bands	Polymorphic bands	% Polymorphism
E_{AAG} X M_{CAA}	48	10	36	75.00
E_{ACA} X M_{CAG}	52	15	40	76.92
E_{AAC} X M_{CTG}	69	22	48	69.56
E_{ACA} X M_{CTC}	61	18	41	67.21
Total	230	65	165	71.73

2.2 Genetic similarity within and among *Phaseolus spp*. genotypes

The AFLP scored data resulting from the four primer combinations were used to calculate the similarity matrices through Dice's genetic similarities coefficient (DSC's) between wild and common bean accessions. AFLPs generated data show that the highest genetic similarity value was 86.7% and the lowest value was 27.7% with an average of 57.2% as shown in (Fig. 4). Based on AFLP analysis, the highest similarity (86.7%) detected by the AFLP assay was between wild tepary bean accessions (G40083 and G40084) both share the same genetic background and geographical region since they were collected from Mexico and southwestern USA.

Meanwhile the lowest percentage of similarity (27.7%) was detected between wild tepary bean accession G40084 and Ferrary bean cultivar.

The topology of the AFLP data based dendrogram was highly similar with that dendrogram constructed based on RAPD data with a few deviations in the values of similarity coefficients. A constructed dendrogram also separated thirteen types of bean into four major clusters, according to the geographic region of wild bean accessions and to classical botanical classification of cultivated forms of bean (Carmenza *et al.*, 2006; Munoz *et al.*, 2004 and Svetleva *et al.*, 2006).

The first cluster contained wild bean accessions collected from Mexico and southwestern USA (G40083 and G40084), while the second cluster contained cultivated forms of bean (Goru and Ferrary), the third cluster, which contained other cultivated forms of bean (Nebraska and Xera). On the other hand, the fourth cluster could be divided into four subclusters common of bean cultivars. Mv309 and Giza-9 are grouped together in the first subcluster, while, cultivars Argus and Amel are grouped together in the second subcluster. The third subcluster contains cultivars Gomi and Sonate and the fourth subcluster contains cultivar Polysta only (Fig. 4).

2.3 Unique markers and fingerprints revealed by AFLPs

All the AFLP primer combinations used detected unique markers (positive or negative). They reveal a total of 26 unique markers comprising 11 negative and 15 positive markers. The highest number of unique markers (11 markers) is obtained with primer combinations of E-**AAG** X M-**CAA**, whereas the lowest number (four markers) is obtained with E-**ACA** X M-**CTC** with molecular weight ranging from 100 to 480 bp (Table 6). The beans types used in this study could be divided into two groups, the first group contained two wild bean accessions, while the other group included cultivated forms of common bean. The highest numbers of unique markers were detected with tepary bean accessions (G40083 and G40084) from the first group; 5.0 and 4.0 unique markers were identified, respectively, from the second group, four of them recorded 3.0 unique markers (Nebraska, Ferrary, Goru and Xera), respectively, while the lowest numbers of unique markers were detected with Gomi, Sonate, Argus, Amel and Giza-9 with only one unique marker. No unique markers were identified with varieties Polysta and Mv309.

Also, the banding patterns obtained by the different AFLP primer combinations yielded unique fingerprints that characterized each genotype. Moreover, nine unique AFLP markers were identified. These markers could be used to differentiate between wild tepary bean accessions and cultivated forms of bean. Seven out of nine markers could be used to differentiate wild type of bean from the other types used in this study (cultivated forms of bean), while two markers could be used to differentiate cultivated forms of bean from wild tepary bean accessions. Two specific AFLP markers are identified for wild tepary bean accessions (G40083 and G40084) with primer combinations of E-**AAG** X M-**CAA**, with molecular weights 220 and 230 bp. The obtained findings are in good accordance with (Singh *et al.*, 1999 and Srivastava *et al.*,2001). Who reported that several rare and accession specific bands were identified. Such specific bands could be effectively used to distinguish the different genotypes of species from different eco-geographic regions, meanwhile, (Zhang *et al.*, 1998) declared that there were no region specific markers identified when assessed genetic diversity in 80 cultivars of sweet potato from four different geographical regions.

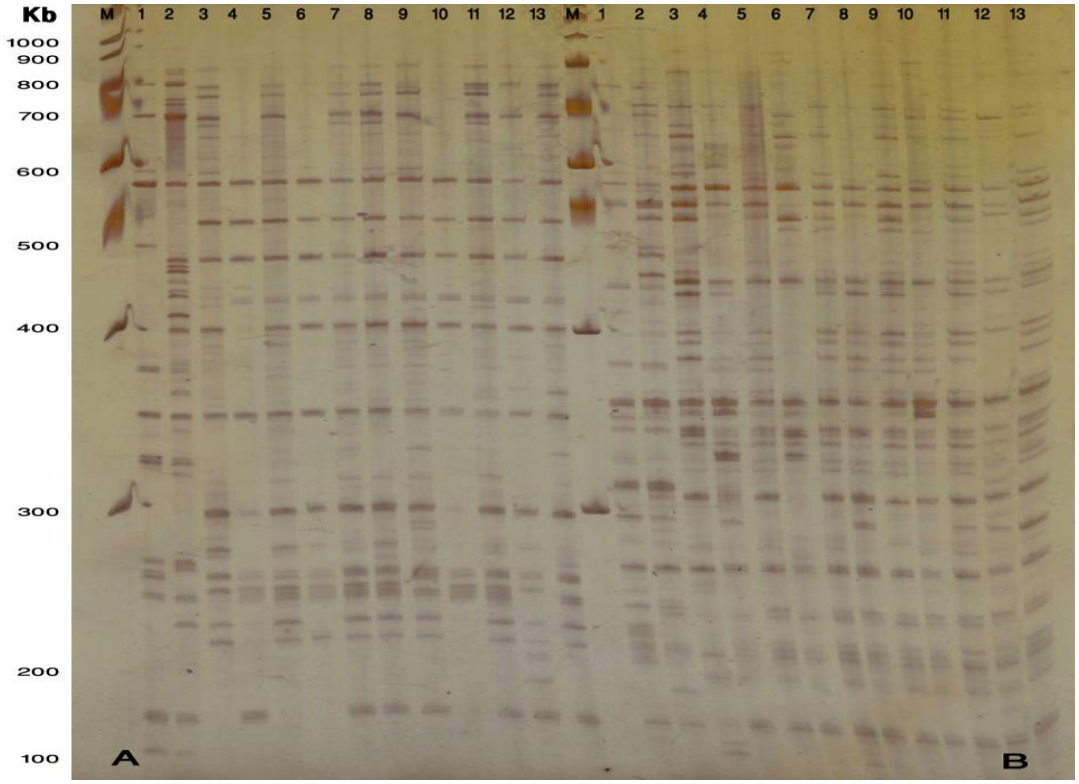

Fig. (3): AFLP profiles of 13 bean genotypes (1 - 13) as revealed by AFLP primer combinations (A) E-AAG X M-CAA and (B) E-ACA X M-CAG. Lanes 1 and 2 represent wild tepary bean accessions (*G40083 and G40084*) and lanes 3 to 13 represent: common bean relatives (Polysta, Nebraska, Gomi, Ferrary, Mv309, Giza-9, Sonate, Goru, Argus, Xera and Amel respectively. DNA molecular weight standards (M)of 100 bp DNA ladder.

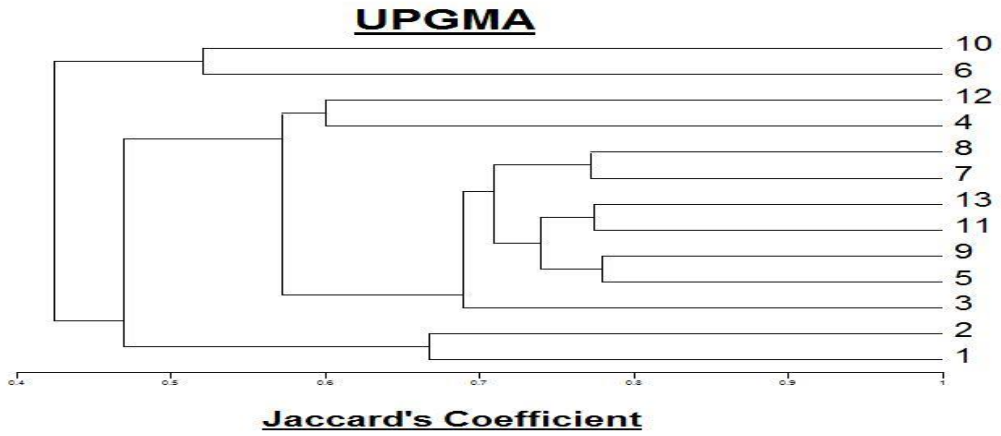

Fig.(4): Dendrogram for the 13 bean accessions constructed from the AFLPs generated data using UPGMA method and similarity matrices computed according to DSC's.

Table (6): Unique positive* and/or negative** AFLP markers, marker size and total number of unique markers revealed from AFLP assayed primer combinations.

No.	Primer combinations	Unique AFLP markers				Total
		UPM*	Size in bp	UNM**	Size in bp	
1	**E_{AAG} X M_{CAA}**	8	110,220,230,310,340, 350,360,520	3	140,150,380	**11**
2	**E_{ACA} X M_{CAG}**	1	220	4	120,130,150,480	**5**
3	**E_{AAC} X M_{CTG}**	4	90, 380, 440, 510	2	180,300	**6**
4	**E_{ACA} X M_{CTC}**	2	240,260	2	100,120	**4**
	Total		**15**		**11**	**26**

3. Comparison between RAPD and AFLP assay in *Phaseolus spp*.

The differences between RAPD and AFLPs reflect the extent of genome coverage and different evolutionary properties. The genomic distribution of RAPD is random, whereas it is reasonable to expect that AFLPs should have complete genome (Nowosielski *et al.*, 2002 and Lanteri *et al.*,2004).

Menezes *et al.* (2004) and Palomino *et al.* (2005) studied the genetic diversity within common bean cultivars using RAPD markers and verified high genetic variability within carioca cultivars that differed from the 'wild tepary beans'. Although RAPD markers have been extensively used for common bean fingerprinting and genetic analysis, AFLP analysis provides a higher level of polymorphism than RAPD. AFLP markers are advantageous because they reveal a larger number of reproducible markers, thereby increasing the probability of identifying polymorphic markers, even among closely related genotypes such as in common beans (Tohme *et al.*, 1996).

Comparisons of the efficiency of RAPD and AFLPs in assessing the genetic diversity of plants have generally shown that AFLP is the preferred technique (Garcia *et al.*, 2004), in a comparison of AFLP, RAPD, RFLP and SSR markers to evaluate genetic diversity in common bean, found that AFLP was the best-suited molecular assay for accurate fingerprinting and assessment of genetic relationships among tropical maize inbred lines.

In conclusion, this is the first study to use powerful molecular markers such as AFLP and SSRs to assess the genetic variability of tepary beans. Other studies of commercial bean genotypes have used RAPD markers, which are less powerful in revealing the extent of genetic diversity. Menezes *et al.* (2004) and Palomino *et al.* (2005) found that AFLP and RAPD and the number of markers they generated were appropriate for assessing the genetic diversity among common bean genotypes. In practice, AFLP markers were easier to use when screening the whole genome for genetic diversity and were more suitable in distinguishing among very closely related genotypes belonging to the same commercial class, such as the common bean and tepary bean genotypes.

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REFERENCES

Benchimol, L.L.; De Campos, T.; Carbonell, S.A.M.; Colombo, C.A.; Chioratto, A.F.; Formighieri, E.F.; Gouvea, L.R.L. and De Souza, A.P. (2007). Structure of genetic diversity among common bean(*Phaseolus vulgaris* L.) varieties of Mesoamerican and Andean origins using new developed microsatellite markers. Genetic Resource Crop Evol., 54:1747–1762.

Buso, G.S.C.; Amara,l Z.P.S.; Brondani, R. P.V. and Ferreira, M.E. (2006). Microsatellite markers for the common bean *Phaseolus vulgaris*. *Mo*l. *Eco*l. Notes, 6:252–254.

Caixeta, E.T.; Bore, M. A. and Kelly, J.D. (2005). Development of microsatellite markers based on BAC common bean clones. Crop Breed. Appl. Biotechnology, 5:125–133.

Campos, T.; Benchimol, L.L.; Carbonell, S.A.M.; Chioratto, A.F.; Formighieri, E.F. and De Souza, A.P. (2007). Microsatellites for genetic studies and breeding programs in common bean. Pesqui Agropecu Bras., 42:589–592.

Cardoso, J.M.K.; Oblessuc, P.R.; De Campos, T.; Sforca, D.A.; Carbonell, S.A.M.; Chioratto, A.F.; Formighieri, E.F.; De Souza, A.P. and Benchimol, L.L. (2008). New microsatellite markers developed from an enriched microsatellite common bean library. Pesqui Agropecu Bras., 43:929–936.

Carmenza, M. L.; Myriam, C. D.; Daniel, G. D. and Matthew, W. B. (2006). Taxonomy of tepary bean and wild relatives as determined by amplified fragment length polymorphism (AFLP) Markers. Crop Sci., (46):1744-1754.

Debouck, D.G. (1999). Diversity in *Phaseolus* species in relation to the common bean. p. 25–52. In S.P. Singh (ed.) Common bean improvement in the twenty-first century. Kluwer Academic Publishers, Dordrecht, Holland.

Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure to small amounts of fresh leaf tissue. Phytochem Bull, 19:11–15.

Driessen, S., Pohl M. and Bartsch, D. (2001). RAPD-PCR analysis of the genetic origin of sea beet (*Beta vulgaris* ssp. maritima) at Germany's Baltic Sea Coast. Basic Appl. Ecol., 2: 341–349.

El-Fiky, Z.A. and Wafaa, M. S. (2002). Characterization of cowpea (*Vigna unguiculata* L.) genotypes based on yield traits and RAPD-PCR analyses. Arab J. Biotech., 6 (1): 67-78.

Franklin, C.J.; Mohammed, M.M.S.; Thomas, G .; Varghese, G .; Selvaraj, N. and Dorai, M. (2009). Genetic diversity and conservation of common bean (*Phaseolus vulgaris* L., Fabaceae) landraces in Nilgiris. Curr. Sci., 97(2): 227-235.

Gaita´n-Solıs, E.; Duque, M.C.; Edwards, K.J. and Tohme, J. (2002). Microsatellite repeats in common bean (*Phaseolus vulgaris*): isolation, characterization, and cross-species amplification in *Phaseolus* spp. Crop Sci., 42:2128–2136.

Garcia, A.A.F.; Benchimol, L.L.; Barbosa, A.M.M.; Geraldi, I.O.; Júnior, C.L.S. and Souza, A.P. (2004). Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. Genet. Mol. Biol., 27:579-588.

Geleta, L.F.; Labuschagne, M.T. and Viljoen, C.D. (2005). Genetic variability in pepper (*Capsicum annuum* L.) estimated by morphological data and amplified fragment length polymorphism markers. Biodivers. Conserv., 14:2361-2375.

Guerra-Sanz, J.M. (2004). New SSR markers of *Phaseolus vulgaris* from sequence databases. Plant Breeding, 123:87–89.

Hanai, L.R.; Campos, T.; Camargo, L.E.A.; Benchimol, L.L.; De Souza, A.P.; Melotto, M.; Carbonell, S.A.M.; Chioratto, A.F.; Consoli, L.; Formighieri, E.F.;Siqueira, M.; Tsai, S.M. and Vieira, M.L.C. (2007). Development, characterization, and comparative analysis of polymorphism at common bean SSR loci isolated from genic and genomic sources. Genome, 50:266–277.

Lanteri, S.; Saba, E.; Cadinu, M.; Mallica, G.M.; Baghino, L. and Portis, E. (2004). Amplified fragment length polymorphism for genetic diversity assessment in globe artichoke. Theor. Appl. Genet. 108: 1534–1544.

Maras M., Sustar-Vozlic J., Javornik B. and Meglic V.(2008). The efficiency of AFLP and SSR markers in genetic diversity estimation and gene pool classification of common bean (*Phaseolus vulgaris* L.) Acta Agric Slov., 91:87–96.

Marechal, R. (1970). Donnees cytologiques sur les especes de la sous tribu des Papilionaceae -Phaseolae-Phaseolinae. Deuxieme serie. Bull. Jard. Bot. Nat. Belge., 40:307–348.

Menezes, M.V.C.O.; Santos, J.B. and Menezes, M. (2004). Genetic diversity in Carioca and Pérola cultivars of common bean based on RAPD markers. Crop Breed. Appl. Biotechnol., 4:178–182.

Monaj, T.; Singh, N.K.; Meenal, R. and Kumar, N. (2005). RAPD markers in the analysis of genetic diversity among common bean germplasm from Central Himalaya. Genetic Resources and Crop Evol., 52: 315-324.

Munoz, L. C.; Blair, M. W.; Duque, M. C.; Tohme, J. and Roca, W. (2004). Introgression in common bean tepary bean interspecific congruity backcross Lines as measured by AFLP markers. Crop Sci., (44):637-645.

Nei, M. and Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., 76: 5269–5273.

Nowosielski, J.; Podyma, W. and Nowosielska, D. (2002). Molecular research on the genetic diversity of Polish varieties and landraces of *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. using the RAPD and AFLP methods. *Cell Mol. Biol. Lett.,* 7:753–762.

Pallottini, L.; Garcia, E.; Kami, J.; Barcaccia, G. and Gepts, P. (2004). The genetic anatomy of a patented yellow bean. Crop Sci., 44:968-977.

Palomino, E.C.; Mori, E.S.; Zimback, L.; Tamborussi, E.V. and Moraes, C.B. (2005). Genetic diversity of common bean genotypes of Carioca commercial group using RAPD markers. Crop Breed Appl. Biotechnol. 5:80–85.

Papa, R. and Gepts, P. (2003). Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. Theor. Appl. Genet., 106:239-250.

Powell, W.; Morgante, M.; Andre, C.; Hanafey, M.; Vogel, J.; Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed., 2:225–238.

Rohlf, F.J. (1993). NTSYS-PC numerical taxonomy and multivariate analysis system. Version 2.02. Exeter Pub., Setauket, N.Y.

Rosales-Serna, R.; Hernandez-Delgado, S.; Gonzalez-Paz, M.; Acosta-Gallegos, J.A. and Mayek-Perez, N. (2005). Genetic relationships and diversity revealed by AFLP markers in Mexican common bean bred cultivars. Crop Sci., 45:1951-1957.

Ruas, P.M.; Bonifacio, A.; Ruas, C.F.; Fairbanks, D.J. and Andersen, W.R. (1999). Genetic relationship among 19 accessions of six species of *Chenopodium* L., by random amplified polymorphic DNA fragments (RAPD). Euphytica, 105: 25–32.

Shen, Y.; Ford-Lloyd, B.V. and Newbury, H.J. (1998). Genetic relationships within the genus Beta determined using both PCR-based marker and DNA sequencing techniques. Heredity, 80: 624–632.

Shen, Y.; Newbury, H.J. and Ford-Lloyd, B.V. (1996). The taxonomic characterization of annual *Beta* germplasm in a genetic resources collection using RAPD markers. Euphytica, 91: 205–212.

Singh, A.; Nagi, M.S.; Rajagobal, J.; Tomar, U.K.; Srivastava, P.S. and Lakshmik, M. (1999). Assessment of genetic diversity in *Azadirachta indica* using AFLP markers. Theor. Appl. Genet., 99: 272–279.

Srivastava, A.; Gupta, V. and Pradhan, A.K. (2001). AFLP based genetic diversity assessment amongst agronomically important natural and some newly synthesized lines of *Brassica juncea*. Theor. Appl. Genet. 102: 193–199.

Svetleva, D.; Pereira, G.; Carlier, J.; Cabrita, L.; Leitão, J. and Genchev, D. (2006). Molecular characterization of *Phaseolus vulgaris* L. genotypes included in Bulgarian collection by ISSR and AFLP analyses. Sci. Hort., 109:198-206.

Thomposn, P.G.; Hong, L.L.; Ukoskit, K. and Zhu, Z. (1997). Genetic linkage of randomly amplified polymorphic DNA (RAPD) markers in sweet potato. J. Amer. Soc. Hort. Sci., 122: 79–82.

Tohme, J.; Orlando-Gonzalez, D.; Beebe, S. and Duque, M.C. (1996).AFLP analysis of gene pools of a wild bean core collection. Crop Sci., 36:1375–1384.

Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; Van de Lee, T.; Hornes, M.; Freijters, A.; Pot, J.; Peleman, J.; Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res., 23: 4407–4414.

Wang, M. and Goldman, I.L. (1999). Genetic distance and diversity in table beet and sugar beet accessions measured by randomly amplified polymorphic DNA. J. Amer. Soc. Hort. Sci., 124: 630–635.

Williams, J.G.K.; Kublick, A.R.; Livak, K.J.; Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531–6535.

Yu, M.A. and Bliss, F.A. (1978). Seed proteins of common bean. Crop Sci., 18:431-437.

Zhang, D.P.; Ghislain, M.; Huamán, Z.; Cervantes, J.C. and Carey, E.E. (1998). AFLP assessment of sweet potato genetic diversity in four tropical American regions. CIP Program Report, pp. 303–310.

Zitoun, B.; De Caraffa, V.B.; Giannettini, J.; Breton, C.; Trigui, A.; Maury, J.; Gambotti, C.; Marzouk, B. and Berti, L. (2008). Genetic diversity in Tunisian olive accessions and their relatedness with other Mediterranean olive genotypes. Sci. Hortic., 115: 416–419.

الملخص العربي

الاختلافات الوراثية لبعض أنواع الفاصوليا البرية و المنزرعة باستخدام الواسمات الجزيئية RAPD , AFLP

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أجريت هذه الدراسة لتقدير مدى قدره الواسمات الجزيئية RAPD , AFLPفى تقدير مستوى الاختلافات الوراثية ضمن و بين مجموعة من اصناف الفاصوليا المنزرعة فى مصر و المستوردة و فاصوليا التبارى البرية و كذلك اجراء مقارنة بين الاختبارين لتقدير مدى مقدرة كلا منهما فى تقسيمهم طبقا للأصل الجينى لمنشئهم. و كانت الشظايا الناتجة من استخدام 12 بادى RAPDو 4 بادى AFLP و قد تم الحصول على 119 شظية من شظايا الدانا من بادئات RAPD و 165 شظية من شظايا الدانا من بادئات AFLP. و قد أوضحت نتائج تحليل بيانات ال RAPD وجود تباين بين انواع و اصناف الفاصوليا المختلفة تتراوح بين 44.6 الى 93.8% بينما أظهرت نتائج تحليل بيانات ال AFLP ان اعلى معدل للاختلافات الوراثية كانت 86.7% بينما كان اقل معدل 27.7% بمتوسط 57.2% و قد تم أجراء تحليل علاقات درجات القرابة و التشابة الوراثية بناء على مصفوفات التشابة المأخوذة من كل نوع من الواسمات الجزيئية باستخدام طريقة ال UPGMA لرسم دندروجرامات توضح القرابة الوراثية.