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Comparative Genomic Studies in Leguminous Species

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Abstract: Comparative Genomics is relatively a new discipline of Genetics, which deals with the relationships between the genomes of genera, species, varieties or strains. For elaborating comparative genomics of legumes; fourteen accessions belonging to seven species viz; *Vigna radiate, Vigna mungo*, *Vicia ervilia*, *Vicia faba*, *Phaseolus vulgaris*, *Cicer arietinum* and *Lens culinaris*, were analyzed using Randomly Amplified Polymorphic DNA (RAPD). Alleles of various sizes ranging from 500-1400 bp were amplified. On an average 3.5 allele per genotype were amplified. The average genetic distance ranged from 4-91%. Phylogenetic relationship among the legumes based upon DNA analysis was studied through dendrogram analysis. All the accessions were clustered in 6 groups. It was found that *Vigna radiata* and *Vigna mungo* accessions were most distantly related to each other.

Key words: Dendrogram, genetic distances, legumes, phylogenetic relationship, randomly amplified polymorphic DNA

INTRODUCTION

Papilionaceae (legumes family) is a very important family of commercial crops all over the world. In Pakistan various kinds of legumes viz; chickpea (*Cicer arietinum* L.), Lentil (*Lens culinaris*), mung beam (*Vigna mungo*), mash (*Vigna radiata*), beans (*Phaseolus vulgaris*) and peas (*Pisum sativum*) are grown. Although area under legumes cultivation has increased in the past few years, for example, area under cultivation of green peas in Pakistan has increased from 2880 thousand ha during 200-2003 to 31031 thousand ha during 2005-2006 (Anonymous, 2007), over all yield of legume crops did not increased substantially over the same period. The national average yield of major legume crops in Pakistan is approximately 0.5 tonnes per ha as compared to the average yield of legumes in the developed countries, for example Australia, which is almost 8 times (5496.7 $Kg.ha^{-1}$) higher than national average of Pakistan (Ali and Randles, 1997).

A prerequisite for increasing yield of legumes is the Comparative Genomics studies which help in better understanding of genome structure (Alonso *et al*., 2003; Adjaye *et al*., 2004; Zhu *et al*., 2005). Previously various kinds of molecular markers including Restriction Fragment Length Polymorphism (RFLP), Polymerase Chain Reaction (PCR), Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphism (SNiPs) have been used for estimation of genetic diversity in various crop species (Rafalski *et al*., 1996; Demeke and Adams, 1994). Among these assays procedures, Polymerase Chain Reaction are relatively easier, cheaper and faster than other assay systems and have been used extensively for the estimation of genetic diversity. Among PCR based assays, Simple Sequence Repeat Primers (SSR) Allele Specific Amplifications (ASA), Sequence Tag Site amplification (STS) etc have been developed and used in breeding programs (Rafalski *et al*., 1996). Relatively recent introduction of Randomly Amplified Polymorphic DNA (RAPD) is more user's friendly as it does not require any sequence information (Williams *et al*., 1990). Present study was undertaken to study comparative genomics of legumes species using RAPD based assays.

MATERIALS AND METHODS

The study was conducted at the Department of Genetics, Hazara University during 2008-2009. During present study, DNA of 14 accessions belonging to seven species of legumes were analyzed using Randomly Amplified Polymorphic DNA (RAPD). Seeds were obtained from Plant Genetic Resource Institute, NARC, and Food Legumes (Pulses) Program, NARC, Islamabad (Table 1).

Total Genomic DNA was isolated using modified small scale DNA isolation procedure (Czaplicki *et al*., 2000; Weining and Langridge, 1991; Doyle and Doyle, 1987). Quality and quantity of the DNA was checked on 1% agarose/TBE gel. Gels were run at constant voltage of 70 volts for approximately one hour and observed under UV light using "Uvitech" gel documentation system. Polymerase Chain Reaction (PCR) was carried using protocol described by Mukhtar *et al*., (2003) and Devos and Gale (1992). Components of PCR reaction were the genomic DNA used as template, dNTPs (dATP,

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Table 1: Species used to study comparative Genomics in Legumes

S.No.	Genus	Species	Accession Nos	Source	Origin
1	*Vigna*	*Radiata*	013986	PGRI	Punjab Pak
2	*Vigna*	*Radiata*	014222	PGRI	Balochistan Pak
3	*Vigna*	*Radiata*	014234	PGRI	Sindh Pak
4	*Vigna*	*Mungo*	013861	PGRI	Punjab Pak
5	*Vigna*	*mungo*	013824	PGRI	Punjab Pak
6	*Vigna*	*mungo*	013938	PGRI	Punjab Pak
7	*Vicia*	*ervilia*	013220	PP	Syria
8	*Vicia*	*Faba*	013244	PP	Balochistan Pak
9	*Phaseolus*	*vulgaris*	018288	PP	NWFP Pak
10	*Phaseolus*	*vulgaris*	018287	PP	NWFP Pak
11	*Cicer*	*arietinum*	017080	PP	Pakistan
12	*Lens*	*culinaris*	01	PP	NIAB Faisalabad(Desi) 2006
13	*Lens*	*culinaris*	02	PP	ARI D.I khan 2004
14	*Lens*	*culinaris*	03	PP	NIAB Faisalabad 2002

PGRI = Plant Genetic Resource Institute Islamabad, PP = Pulses program, NARC. Islamabad

Table 2: List of RAPD primers used for estimation of genetic diversity in 14 accessions of legumes used during present study

S. No	Oligo name	Sequence (5'-3')	Mol. Wt	%GC
1	GLA-14	TCTGTGCTGG	3050	60
2	GLA-16	AGCCAGCGAA	3040	60
3	GLB-16	TTTGCCCGGA	3108	60
4	GLD-03	CTCGCCGTCA	3003	70

dCTP, dGTP and dTTP), Random primer, Taq Polymerase buffer, $MgCl_2$ and Taq DNA Polymerase. Four Randomly Amplified Polymorphic DNA (RAPD) primers viz; GLA-14, GLA-16, GLB-16 and GLD-03 (obtained from Gene-Link, Inc, USA) were used to study genetic differences at DNA level in the material. Sequence and molecular weight of the primers are presented in Table 2.

For statistical analysis, every band was considered as single locus/allele. Alleles/loci were scored as present (1) or absent (0). Bivariate 1-0 data matrix was generated and genetic distances (GD) among the genotypes were estimated using Unweighted Pair Group of Arithmetic Means (UPGMA) as described by Nei and Li (1979). The formula used to calculate GD is given below

$$GD=1-d_{xy}/d_x+d_y-d_{xy}$$

Where GD = Genetic distance, d_{xy} = Total number of common bands in two genotypes, dx = Total number of bands in genotypes # 1 and d_y = Total number of bands in genotype #2. Computer program "Pop gene 32" was used to construct Dendrogram using bivariate 1-0 data matrix.

RESULTS AND DISCUSSION

Total genomic DNA from 14 accessions of the six legumes species viz; *Vigna radiata (*Acc Nos 013986,014222,014234i), *Vigna mungo* (Acc Nos 013861,013824,013938i), *Vicia ervilia* (Acc No 013220i), *Vicia faba* (Acc No 013244i), *Phaseolus vulgaris* (Acc No 018288), *Cicer arietinum* (Acc No 017080), *Lens culinaris* (Acc Nos 01,02,03) was isolated using small scale DNA isolation procedure. An example of PCR amplification of Legumes accessions using RAPD primer GLB-16 are presented in Fig. 1.

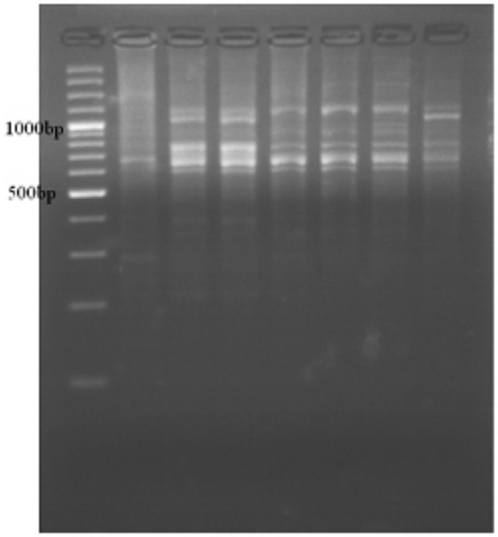

Fig. 1: PCR amplification profile of 7 legume accessions using RAPD primer GLB-16. M= Molecular weight marker (in bp) Is presented on left.
1 = *Vicia faba* Acc No 013244, 2 = *Phaseolus vulgaris* Acc No 018288, 3 = *Phaseolus vulgaris* Acc No 018287, 4 = *Cicer arietinum* Acc No 017080, 5 = *Lens culinaris Acc No* 01, 6 = *Lens culinaris Acc No* 02, 7 = *Lens culinaris Acc No* 03

Average genetic distance estimates among the 14 legume accessions ranged from 4-91% (Table 3). Maximum average genetic distance (GD = 91%) was estimated for 1 comparison viz; *Vigna radiata* Acc No 013986 - *Cicer arietinum* Acc No 017080. One comparison (*Vigna mungo* Acc No 013861 - *Vigna mungo* Acc No 013938) showed almost complete homozygosity (average GD = 4%). Rest of the comparisons showed varying range of

Table 3: Average estimates of Genetic distances among 14 legume accessions using four RAPD primers (GLA-14, GLA-16, GLB-16 and GLD-03)

	1	2	3	4	5	6	7	8	9	10	11	12	13
1													
2	0.25												
3	0.14	0.12											
4	0.81	0.39	0.49										
5	0.74	0.55	0.49	0.11									
6	0.71	0.35	0.49	0.04	0.14								
7	0.72	0.46	0.39	0.25	0.20	0.24							
8	0.58	0.59	0.48	0.55	0.58	0.54	0.56						
9	0.44	0.53	0.44	0.60	0.58	0.56	0.67	0.37					
10	0.44	0.52	0.44	0.57	0.64	0.52	0.61	0.43	0.09				
11	0.91	0.59	0.61	0.68	0.61	0.64	0.56	0.39	0.37	0.41			
12	0.88	0.61	0.56	0.52	0.68	0.69	0.53	0.61	0.40	0.42	0.35		
13	0.51	0.61	0.58	0.68	0.66	0.67	0.50	0.47	0.47	0.49	0.35	0.14	
14	0.65	0.63	0.61	0.56	0.56	0.56	0.36	0.58	0.48	0.51	0.58	0.25	0.23

1 = *Vigna radiata* Acc No 013986, 2 = *Vigna radiata* Acc No 014222, 3 = *Vigna radiata* Acc No 014234, 4 = *Vigna mungo* Acc No 013861, 5 = *Vigna mungo Acc No* 013824, 6 = *Vigna mungo* Acc No 013938, 7 = *Vicia ervilia* Acc No 013220, 8 = *Vicia faba* Acc No 013244, 9 = *Phaseolus vulgaris* Acc No 018288, 10 = *Phaseolus vulgaris* Acc No 018287, 11 = *Cicer arietinum* Acc No 017080, 12 = *Lens culinaris Acc No* 01, 13 = *Lens culinaris Acc No* 02, 14 = *Lens culinaris Acc No* 03.

average genetic distances but in most of the comparisons (63 comparisons) average genetic distance distances were moderate (average GD ranging from 40-70%).

The data obtained during present study using four RAPD primers were used to analyze phylogenetic relationship among the legume accessions. Computer program "Popgene ver. 32" was used to construct the dendrogram (Fig. 2). The fourteen acccessions were clustered in 6 groups "A", "B" "C' "D" "E" and "F" comprising 3, 2, 2, 1, 3 and 3 accessions, respectively. Groups A, B, C, D, E and F comprised *Vigna radiata, Vicia (vicia ervilia* and *Vicia faba), Phaseolus vulgaris, Cicer arietinum, Lens Culinaris* and *Vigna mungo*, respectively. It was found that *Vigna radiata* and *Vigna mungo* were most distantly related to each other on the basis of data obtained using Randomly Amplified Polymorphic DNA primers. It was also observed that differences within *Vigna mungo* accessions were less than that observed for accessions within *Vigna radiata* (Fig. 2). Similarly differences within the two accessions of *Phaseolus vulgaris* were minimum among all the comparisons studied during present research work.

During present study PCR, alleles of various size (ranging from 100-1400 bp) were amplified. On an average 3.5 allele per genotype were amplified. Similar results were reported by Mukhtar *et al*., (2003), Hamza *et al*., (2004) and Fu *et al*., (2006) using PCR based assays. Although a wide level of genetic diversity was observed during present study (average genetic distance estimates using the four RPAD primers ranged from 4-91%), but most of the comparisons (63 comparisons) showed moderate average genetic distance estimates (average GD ranging from 40-70%). These results further strengthened previous findings by Ghafoor *et al*. (2005), Asghar *et al*. (2003), Choi *et al*. (2004), Ferreira *et al*. (2000) and Dasgupta and Singh (2003) who reported low to medium level of genetic diversity in various legume species.

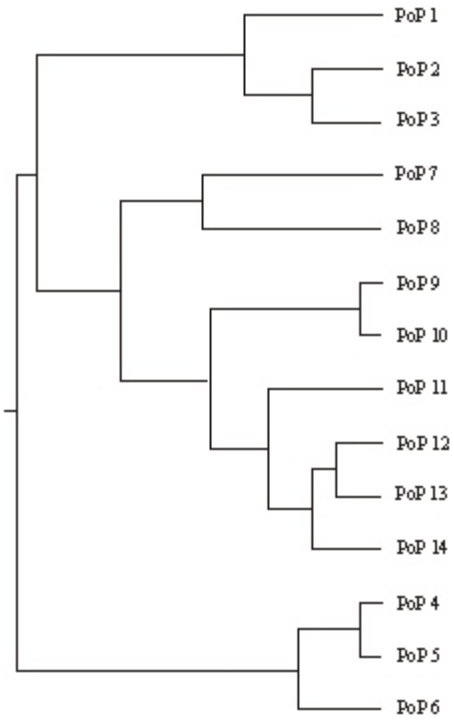

Fig. 2: Dendrogram constructed for 14 legume accessions based on data obtained using four Randomly Amplified Polymorphic DNA primers.
1 = *Vigna radiata* Acc No 013986, 2 = *Vigna radiata* Acc No 014222, 3 = *Vigna radiata* Acc No 014234, 4 = *Vigna mungo* Acc No 013861, 5 = *Vigna mungo Acc No* 013824, 6 = *Vigna mungo* Acc No 013938, 7 = *Vicia ervilia* Acc No 013220, 8 = *Vicia faba* Acc No 013244, 9 = *Phaseolus vulgaris* Acc No 018288, 10 = *Phaseolus vulgaris* Acc No 018287, 11 = *Cicer arietinum* Acc No 017080, 12 = *Lens culinaris Acc No* 01, 13 = *Lens culinaris Acc No* 02, 14 = *Lens culinaris No* 03.

Phylogenetic relationship observed during present studies revealed that *Vigna radiata* and *V. mungo* though belonging to the same genera, were more distantly related in terms of phylogenetics as compared to other genera. Differences within *Vigna mungo* accessions were less than that observed for accessions within *Vigna radiata*. Similarly differences within the two accessions of *Phaseolus vulgaris* were least among all the comparisons. As very low level of genetic variability was observed within *Vigna* sp. (which are important legumes of Pakistan), it is suggested that breeding program aimed at increasing genetic diversity with in legumes should be launched and those programs may involve wide hybridization (for example crossing between *Vigna radiata* and *Vigna mungo* accessions). It is also suggested that more studies of similar nature should be conducted for better understanding of the genome structure of legumes, which will ultimately help designing better strategies for legume improvement in Pakistan.

CONCLUSION

It is concluded that the legume species commonly grown in pakistan have sufficient amount of genetic diversity which can be used for the imporvement of legume crops in the country through hybridization. It has also ben conlcuded that *Vigna radiata* and *V. mungo* though belonging to the same genera were parted more distantly in terms of phylogenetics as compared to other genera.

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