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RESEARCH ARTICLE

GENETIC DIVERSITY EVALUATION IN PIGEON PEA [*Cajanus cajan* (L.) Millsp.] USING PROTEIN PROFILING AND RAPD

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ABSTRACT

Protein profiling and RAPD of five accessions (Amar, Azad, MAL-13, NDA-1, and Pusa-9) of *Cajanus cajan* were investigated to evaluate the genetic diversity. The present protein profile revealed that experimental accession Amar (A1) is very close to the accession Pusa-9(A5) and accessions MAL-13 (A3) and NDA-1(A4) are closer at molecular level as compared to other accessions. . A dendrogram constructed based on UPGMA clustering method revealed two major clusters, cluster -1 and cluster-11 comprising of two accessions each. The accession Azad (A2) occupies a distinct place as revealed in dendrogram .In RAPD similar results were observed .The polymorphism percentage in protein profiling was 90.09% and in RAPD 80% polymorphism was observed.

INTRODUCTION

Pigeon pea [*Cajanus cajan* (L.) Millsp.] Is one of the major grain legume (Pulse) crops of tropics and sub tropics. The Indian subcontinent, accounts for about 90% of the global production. Its seed protein content is (approximately 21%) is also well comparable with that of other major grain legumes. . Genetic diversity of seed proteins has been reported for many crop. Genetic diversity refers to any variation in the nucleotides, genes. Chromosomes or genomes of organism. Thus each gene comprises a hereditary section of DNA that occupies a specific place of the chromosomes and controls a particular characteristic of an organism..Each allele codes for the production of amino acids that string together to form proteins .Thus differences in the nucleotide sequence of alleles result in production of slightly different strings of amino acids or variant forms of proteins code for the development of anatomical and physiological characteristic of an organism which are also responsible for determining aspects of the behavior of the organism .In recent years SDS-PAGE of total seed proteins and cytological analysis has been found wide application in resolving genetic diversity and for intra and interspecific studies.There has been substantial number of studies that have used sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to profile seed storage proteins in legumes. Randomly amplified polymorphic DNA (RAPD) markers have been used for numerous applications in plant molecular genetics research despite having disadvantages of poor reproducibility. Being a multilocus marker with a simplest and fastest detection technology have been successfully employed for determination of intervarietal genetic diversity in several grain legumes. The aim of present study was to find out genetic diversity in five accessions of pigeon pea namely, Amar, Azad, MAL-13, NDA-1, pusa-9 using protein profiling and RAPD. Knowledge and molecular relationships between plant accessions is very useful in planning effective breeding strategies designed to transfer desirable genes or gene clusters from one accession to another, Thereby producing fruitful genomic reconstructions and disease free plants. Determining of genetic diversity of any given crop accessions is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of breeding scheme.

MATERIALS AND METHODS

Materials for present study were collected from Indian Institute of Pulse Research (IIPR) Kanpur (U.P.) India, to study genetic diversity based on SDS-PAGE and RAPD. Total seed proteins were extracted from 1g of seed flour (Lamelia, 1970) using 400 µl of extraction buffer that contained 25Mm trisHCL pH-8.3, 12% SDS.5M urea and 10% mercapto-ethanol. Seed flour was thoroughly mixed with buffer by vertexing. The extracted protein was separated by centrifuging the sample at the rate of 1500rpm for 10 minutes. Electrophoresis was carried out in discontinuous SDS-PAGE using 7% acryl amide gels. Electrophoresis was run at 50v.The gels were stained in the staining solution containing 40ml methanol, coomassie brilliant blue [1%] 1g and glacial acetic acid (10ml) was made up to 100ml by adding distilled water. Destaining was done in a solution containing 30ml methanol, 6ml glacial acetic acid, 74 ml of distilled water until the background color disappeared, and protein bands were clearly visible.

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Genomic DNA isolation: Genomic DNA was isolated from seeds using CTAB based method, analysed and quantified by standard methods

PCR amplification using RAPD primer: One ligonucleotide primer selected from available literature were synthesized from Bangalore Genei, India private limited.PCR was carried out in 25µl of 10 x Taq assay buffer (Tris with 15Mm $Mgcl_2$), 10Mm of each Dntp, 1U Taq polymerase,2µl of primer 2µl of Template DNA. Amplification was carried out in thermo cycler programmed for 40 cycles with an initial denaturation of 95^0c for five minutes followed by cycling conditions of denaturation at 92^0c for 1 minute , annealing at 3 min at 35^0c and extension at 72^0c for 2 min ,After 40 cycles, there was a final extension step of 3 min at 72^0c.The amplicons where analysed on 2% agarose gels and detected by staining with ethidium bromide. UV –illuminated gels were photographed with gel documentation system.

Data analysis: Protein bands were scored depending on their presence [1] and absence [0]. Similarity coefficient was determined and hierarchical clustering was constructed by unweighted pair group method with arithmetic average [UPGMA].The computer software PHYLIP_1 was used for this purpose.The amplification products were scored for each accession because of presence and absence of band.i. e use of binary code 1 and 0 for the presence or absence of band, respectively. Molecular size (bp) of amplified DNA fragment was derermined by 100- 300bp ladder.DNA fragment analysis were performed using the SPSS 12.0 computer software.

Observations

Protein profiling: For protein profiling, the molecular weights of marker in K.D. were: 97 for first band, 66 for second band, 43 for third band, 29 for fourth band, 14 for fifth band, 9 for sixth band. Distance travelled by tracking dye (Loading dye) was calculated by scale that was equal to 8 cm.

RAPD: For RAPD high polymorphism decamer was selected for evaluating genetic diversity and distance travelled by dye was calculated by scale and it was 9cm. Dendrogram based on UPGMA in five accessions of pigeon pea

RESULTS AND DISCUSSION

Significant variations were observed in biochemical character and molecular characters among five experimental accessions of *Cajanus cajan* belonging to family Fabaceae. To find out diversity among five accessions of pigeon pea biochemical and molecular analysis was done during present investigation. To find out intervarietal correlation between cultivars, several earlier workers e.g., Jha and Ohri and Ladzinsky made protein profiling study through SDS-PAGE and found almost same observations. Present investigation revealed that protein profiling is one of the basic and reliable methods to detect intervarietal genetic diversity and study phyllogenetic relationship among the five selected experimental accessions of *Cajanus cajan* Seed protein pattern can also be used as a promising tool for distinguishing cultivars of particular crop species Jha and Ohri (1996). The SDS is considered to be practical and reliable method for species/varieties identification Gepts . During protein profiling

Table 1. The presence and absence of bands in different accessions of pigeon pea

Band Number	Rf value	Mol.Wt	A_1	A_2	A_3	A_4	A_5
1	0.01	99	+	+	+	+	+
2	0.02	98	-	+	+	+	+
3	0.03	97	+	+	+	+	+
4	0.05	95	+	+	-	+	-
5	0.06	94	+	+	+	+	+
6	0.07	93	+	-	-	-	-
7	0.08	92	+	-	-	-	-
8	0.12	88	-	-	+	+	-
9	0.15	85	+	+	-	-	-
10	0.16	84	-	-	-	-	+
11	0.17	83	-	-	+	+	-
12	0.18	82	+	-	-	-	-
13	0.21	79	-	-	-	+	-
14	0.22	78	-	-	+	-	-
15	0.23	77	-	+	-	-	-
16	0.25	75	-	-	-	+	-
17	0.27	73	+	-	-	-	-
18	0.3	70	-	+	+	+	-
19	0.31	69	+	+	+	-	+
20	0.37	63	+	+	+	-	-
21	0.4	60	-	-	-	-	+
22	0.47	53	-	+	-	-	-
23	0.5	50	+	-	+	+	+
24	0.53	47	-	+	-	-	-
25	0.56	44	+	-	-	-	+
26	0.57	43	-	-	-	+	-
27	0.6	40	-	-	+	-	-
28	0.61	39	-	+	-	-	-
29	0.63	38	-	-	+	-	-
30	0.67	33	-	-	+	-	-
31	0.73	27	-	-	-	+	-
32	0.75	25	-	+	-	-	-
33	0.78	22	-	-	+	-	-

Where A_1=Amar,A_2=Azad,A_3=MAL-13,A_4=NDA-1,A_5=Pusa-

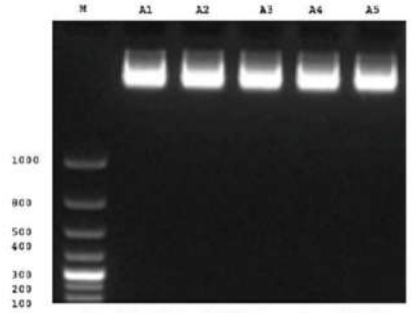


Fig-1 Genomic DNA of five accessions of Cajanus cajan

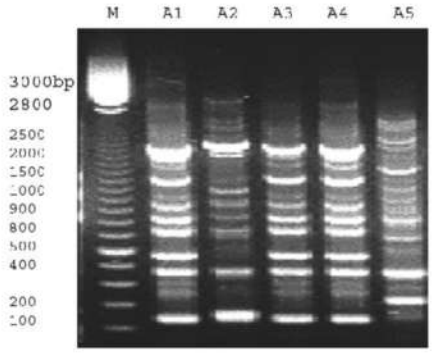


Fig-2 RAPD profile of five accessions of Cajanus cajan generated by using 16ES10C27 primer

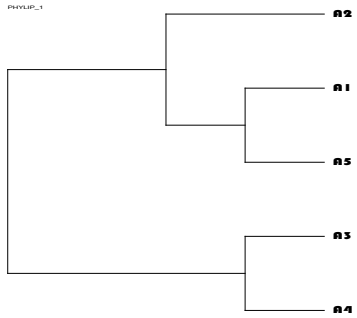
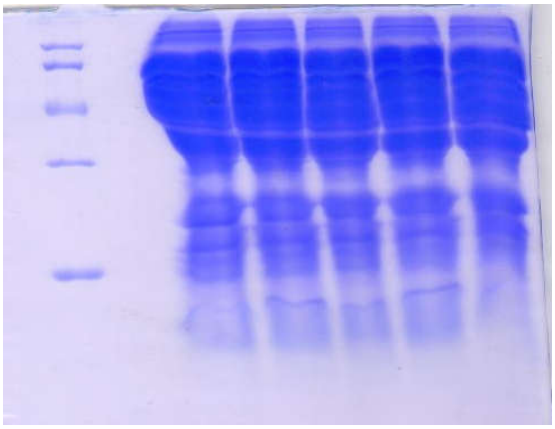


Fig.3. Dendrogram based on UPGMA showing genetic relationship among five accessions of *Cajanus cajan* based on Dice's similarity coefficient estimated for RAPD data



A1 = Amar, A2 = Azad, A3 = MAL-13, A4 = NDA-1, A5 = PUSA-9

Fig. 4: Protein profile of five different accessions of *Cajanus cajan*

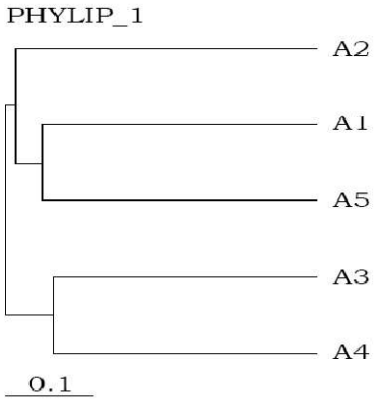


Fig. 5. Dendogram based on UPGMA showing genetic relationship among five accessions of *Cajanus cajan* based on jaccard's similarity, estimates of protein profiling.

of experimental accessions number of bands generated in different accessions of pigeon pea. The protein band for highest molecular weight (i.e., 99 KD) in all accessions of pigeon pea and lowest molecular weight (i.e., 22 KD) was generated in accessions mal-13. When bands of all accessions were compared we got a total of 33 bands. Out of them 30 were polymorphic with a total of 90.09% polymorphism and three bands were monomorphic with 9.09% monomorphism (Table 1). Ghaffor and Arshad [26] got

Table 2. Jaccard's similarity coefficient

	A1	A2	A3	A4
A1				
A2	0.35000			
A3	0.27273	0.31818		
A4	0.23810	0.28571	0.40000	
A5	0.37500	0.27778	0.3333	0.29412

Table 3: Table showing Presence and absence of bands in RAPD profile

Band Number	Rf value	NO. of base pairs	A_1	A_2	A_3	A_4	A_5
1	0.17	2400	+	-	-	-	-
2	0.25	2250	-	+	-	-	-
3	0.26	2220	+	-	-	+	-
4	0.32	2060	-	-	-	-	+
5	0.33	2010	-	+	-	+	-
6	0.36	1920	-	+	-	-	-
7	0.38	1860	-	-	-	-	+
8	0.4	1800	+	+	+	+	+
9	0.42	1740	-	+	-	-	-
10	0.46	1620	-	-	+	+	-
11	0.47	1590	+	-	-	-	-
12	0.48	1560	-	-	-	-	+
13	0.51	1470	+	+	+	+	+
14	0.55	1350	-	+	-	-	+
15	0.56	1320	-	-	+	-	-
16	0.57	1290	+	-	-	+	-
17	0.58	1260	+	+	-	-	+
18	0.6	1200	-	-	+	-	-
19	0.61	1170	-	-	-	+	-
20	0.65	1050	+	+	+	+	+
21	0.68	960	+	+	+	+	+
22	0.72	840	-	-	-	-	+
23	0.75	750	+	-	+	+	-
24	0.77	690	+	-	-	-	-
25	0.81	570	+	+	+	+	+
26	0.86	420	-	-	+	-	-
27	0.88	360	+	-	-	-	+
28	0.9	300	-	-	+	+	-
29	0.92	240	+	-	-	-	+
30	0.98	60	+	+	+	+	+

where A_1=Amar, A_2=Azad, A_3= MAL-13, A_4 = NDA-I, A_5 = PUSA-

Table 4. Dice's similarity coefficient

	A1	A2	A3	A4	A5
A1					
A2	0.35000				
A3	0.35000	0.33333			
A4	0.47368	0.38889	0.56250		
A5	0.45000	0.44444	0.30000	0.28571	

a total of 25 bands and among them 20 were polymorphic with a total of 80% polymorphism.. Similarly kakaei and kahrizi got 17 bands with high polymorphism. Shrivastava and Gupta [28] almost got same results. This little change is might be due to crop change. Jaccards similarity coefficient ranged from 0.23810 to 0.40000 (Table 2). Dendrogram was constructed based on unweighted pair group method using arithmetic averages. Cluster analysis of data placed five accessions of pigeon pea into three clusters. Cluster first comprised two accessions (Amar and Pusa-9); Cluster second comprised two accessions (MAL-13 and NDA-1). Cluster third consisted of only one accession Azad which is placed separate in dendrogram. The number of amplified fragment sizes ranged from 60 – 2400bp. When bands of all accessions were compared we got a total of 30 bands. Out of them 24 were polymorphic with a total of 80% polymorphism and six bands were monomorphic with a total of 20% monomorphism. (Table 3). Almost similar results were observed by Malvia

et al. (2010) .She observed a total of 14 bands and out of them 11 bands were polymorphic with a total of 78.57% polymorphism and 3 bands were monomorphic with a total of 21.42% monomorphism. The little variation observed in polymorphism percentage might be due to the cultivar change. Similarly Mahmood *et al*. (2011) got sixty three bands from seven random primers. Out of sixty three bands fifty were polymorphic in all genotypes. Jan *et al.* (2011) got ninety five RAPD fragments of which ninety were polymorphic with 94.73% polymorphism. The change is might be due to the number of primers used. The other earlier workers viz Chaudhary *et al.* (2008) got 73.7% polymorphism in pigeon pea cultivars and Ratnaparkhe *et al.* (1995) got high polymorphism in wild species as compared to cultivated species.. The other earlier workers viz Sudupak *et al.* (2002), Agrama and Toinstra (2003), Iqbal *et al.* (2010), Deshmukh *et al.* (2009) got almost same results. The change in polymorphism percentage and number of bands is might be due to the different number of primers used and crop change. Dice's similarity coefficient ranged from 0.28571 to 0.47368. (Table 4). Dendrogram was constructed based on unweighted pair group method using arithmetic averages. Cluster analysis of data placed five accessions of pigeon pea into three clusters. Cluster first comprised two accessions (MAL-13 and NDA-1); Cluster second comprised two accessions (Amar and NDA-1). Accession Azad is placed separate in dendrogram (Fig. 3).

Conclusion

The presence of diversity is important for improving any crop accession. An understanding of the magnitude and patterns of genetic diversity in crop plants has important implications in breeding programmes and for conservation of genetic resources. Large numbers of cultivars utilizing limited genetic resource is grown and are being released. Often plant breeders limit their efforts to narrow range of adapted lines of genetic improvement which were more likely to produce economic gains in the short term but have enhanced vulnerability to insect pests and other biotic stresses. Therefore, it is concluded that except Azad, all the experimental accessions show low genetic diversity in central India region and should be diversified using modern breeding techniques. There has been great advancement in marker technology with advent of different DNA markers like Restriction fragment length polymorphism (RFLP), Amplified fragment length polymorphism (AFLP) etc; still RAPD is quite reliable for the evaluation of genetic diversity by using maximum number of random primers for samples provided. The present work carried out with high polymorphism decamer selected indicating genetic diversity in five accessions of pigeon pea could be evaluated further by increasing number of random decamers

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Author's Contributions

The work is original and it has been carried out by SAS for M.Phil. degree under supervision of RK at Bundelkhand University, Jhansi(U.P.), India, and at Indian Grassland of Fodder Research Institute (IGFRI), Jhansi (U.P.) India and Indian Biotechnology research center (IBRC), Agra.

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