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RESEARCH ARTICLE

ANALYSIS OF GENETIC DIVERSITY IN LENTIL (*LENS CULINARIS*) USING CYTOLOGICAL CHARACTER AND PROTEIN PROFILING

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ABSTRACT

Cytological character and protein profile of five accessions (IPL-406, IPL-81, DPL-62, DPL-15, and Sehore 74-3) of lentil (*Lens culinaris*) were estimated through polyacrylamide gel electrophoresis and using cytological characters viz., mitotic index. The present protein profile revealed that experimental accession IPL-406(L-1) is very close to to the accession Sehore-74-3(L-5) and DPL-62(L-3) and DPL-15 (L-4) are more close at molecular level as compared to other accessions. The greatest similarity coefficient (36.3%) was observed between IPL-406 and Sehore-74-3, while lowest similarity (15%) was observed between IPL-406 and DPL-15.At protein profile level a total of 46 protein bands were obtained, 44 protein bands show polymorphism (95.6%) and rest 2 bands show monomorphism (4.3%). Present study has also revealed that the mitotic index was highest in IPL-81 accession (L-2) i.e., 26.71% meaning that the accession has high power of division. The accession IPL-81 show highest germination percentage in first 24th hour and this accession also has highest content of protein i.e., 29.5% than accessions estimated.

INTRODUCTION

Lentil is a self pollinated diploid (Alabboud *et al*., 2000), selender, annual bushy, much branched and herbaceous plant.It is a cool season grain legume with relative genome of 41063 Mbp (Arumuganthan and Earle, 1991). Lentil is an important seed legume crop cultivarted world wide as human food (Ertugrul *et al.,* 2004).It belongs to family Fabaceae. This crop contains 26% 0f protein content. Lentils are always on the list of production as one of the important rabi crops**.** The plant likely originated in the Near East and is believed to have originated from *lens orientalis.* It has been part of human diet since the aceramic (non pottery producing) Neolithic times, being one of the first crops domesticated in the Near East and is an important part of diet in many parts of the world, especially in the Middle East the Indian sub continents(Ahmad *et al*.1996). The ancient pulse crop was domesticated in the Fertile Crecent where it has been cultivated since at least the seventh century B.C. (Ladizinsky, 1979). Indian production of this crop hovers around 10 lakh metric tons per year that cultivated on about 14 lakh hectares of land.Around 90% of the production comes the Eastern and the Western part of the country. Canada is the largest exporter of lentil in the world contributing about 1043200 tonnes / year.

Among biochemcal techniques SDS-PAGE is widely used due o its simplicity and effectiveness for describing the genetic structures of crop gerplasm (Murphy *et al*., 1990 ; Javaid *et al*., 2004 ; Anwar *et al.,* 2003). The main objective of our research was to estimate the potential of SDS-PAGE technique to assess genetic diversity and relatedness among 5 accessions of *Lens culinaris* based on seed storage protein profile and to develop an optimized and efficient operational system for their use. Knowledge of cytological and molecular relationships between plant accessions is very useful in planning effective breeding strategies to transfer desirable genes or genes clusters from one species to another, thereby producing fruitful genomic reconstructions and disease free plants. Estimation of genetic diversity of any crop species is suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a breeding scheme. Genetic diversity refers to any variation in the nucleotides, genes, chromosomes or genome of the organisms. Thus, each gene comprises a hereditary selection of DNA that occupies a specific place of the chromosomes and controls a particular characteristic of an organism (Welsh *et al.,* 1990). Each allele codes for the production of aminoacids that string together to form proteins. Thus, differences in the nuclotide sequence of alleles result in the production of slightly different strings of amino acids of the proteins. These proteins code for the development of the anatomical and physiological characteristics of the organisms (Shibata, 2005).

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MATERIALS AND METHOD

Materials (lentil accessions) for the cytological and protein profiling studies were collected from IIPR, Kanpur (U.P), India. Mitotic index was determined from the root tips of experimental species, root tips were harvested and fixed in 3:1

ratio of alcohol and glacial acetic acid after 6 hours of fixation the root tips were preserved in 70% alcohol.

Formula used:

$$\text{Mitotic index} = \frac{\text{Total no of dividing cells}}{\text{Total no of cells studied}} \times 100$$

Total seed proteins were also extracted from 1g seed flour using 400 micro litre of extraction buffer that contained 25 Mm tris HCl 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 1500 rpm for 10 minutes electrophoresis was carried out in discontinuous SDS-PAGE using 7% acrylamide gel. Electrophoresis was run at 50v. The gels were stained in the staining solution containing 40ml methanol, coomassie blue 1% (1g) and galacial acetic acid (10ml) was made up to 100ml by adding distilled water. Distaining was done in a solution containing 30ml methanol, 6ml glacial acetic 74ml of distilled water until the back ground colour disappeared, and protein bands were clearly visible.

Data analysis

The results obtained from the cytological character were analysed statistically. Rf value and the molecular weight of each protein band was determined (table-7 and -8).Protein bands were scored depending on their presence (+) and absence (-). Similarity coefficient was calculated and dendrogram was constructed by unweighted pair group method with arithmetic averages (UPGMA).

Mitotic index

Mitotic index in different investigated accessions is given in Table-1, determined by the formula described in methods. Protein prifling: For protein profiling , the molecular weights of the marker in K.D. were 97.4 for first band ,66.0 for second band, 43 for third band , 29.0 for fouth band , 20. 1 for fifth band and 14.1 for sixth band. Distance travelled by tracking dye was calculated by scale that equal to 8 cm.

Mitotic study

The mitotic index in different investigated genera is as follows determined by the formula as described in materials and methods is as follows:

S. No.	L1	L2	L3	L4	L5
1	26.7%	9.52%	25.64%	16.54%	26.21%

$$\text{Mitotic index} = \frac{\text{Total no of dividing cells}}{\text{Total no of cells studied}} \times 100$$

Where,

$$\text{Similarly index} = \frac{\text{Total number of similar bands}}{\text{Total number of bands}} \times 100$$

Table 3: Showing presence and absence of bands in protein profile

S.No.	Rf value	Molecular wt.	L1	L2	L3	L4	L5
1.	0.01	99	-	-	+	-	-
2.	0.025	98	+	-	-	-	+
3.	0.03	97	-	-	+	+	-
4.	0.05	95	+	-	+	-	+
5.	0.06	94	+	+	+	+	+
6.	0.08	92	+	-	-	+	+
7.	0.1	90	-	+	-	-	-
8.	0.11	89	+	-	-	-	-
9.	0.12	88	-	+	+	-	-
10.	0.13	87	+	-	-	-	+
11.	0.25	75	+	+	+	+	+
12.	0.26	74	+	-	-	-	-
13.	0.35	65	-	-	+	+	-
14.	0.37	63	-	-	+	+	-
15.	0.4	60	+	+	-	-	+
16.	0.42	58	+	-	-	-	+
17.	0.46	54	+	-	-	-	-
18.	0.56	44	+	+	+	-	+
19.	0.6	40	-	-	-	+	-

Table 4. Showing Jaccard's similarity coefficient

	L1	*L2*	*L3*	*L4*	*L5*
L1	*0.000*				
L2	*0.28571*				
L3	*0.23529*	*0.36364*			
L4	*0.18750*	*0.18182*	*0.45455*		
L5	*0.83333*	*0.33333*	*0.26667*	*O.21429*	*0.000*

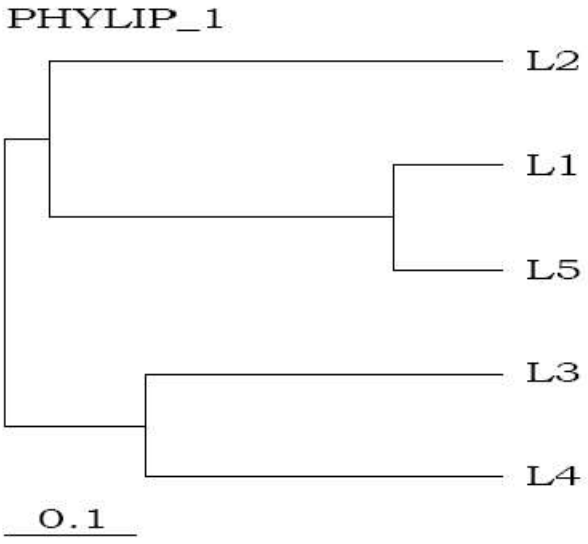
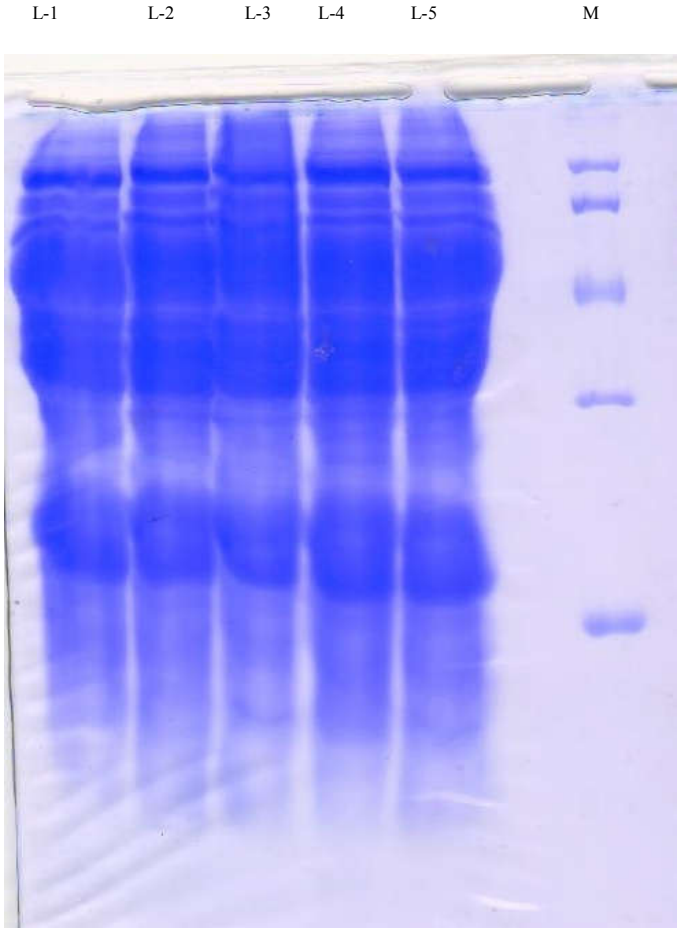


Fig. 1. UPGMA & Jaccard's method dendrogram showing the genetic relationship among 5 accessions of lentil revealed by SDS PAGE cluster analysis

RESULTS AND DISCUSSION

Great variations were observed in qualitative, biochemical and characters among five experimental accessions of *Lens culinaris* belongs to family Fabaceae. To find out diversity among accessions of lentil, cytological and molecular analysis through protein profiling revealed correlation between five experimental accessions of *Lens culinaris.* Cytological study was done to find out mitotic index in different accessions of lentil. The mitotic index was highest in IPL-81 i.e., 26.7% and next in Sehore-74-3 i.e., 26.2% ,DPL-62 i.e., 25.6% ,

IPL-406 i.e., 16.54% , and lowest mitotic index in DPL-15 i.e., 9.52%.



Molecular weight of marker(M):
97.4, 66, 43, 29, 20.1, 14.1 respectively(From top to bottom).

Fig. 2: Photograph shows protein profile of five accessions of lentil(*Lens culinaris*).

Earlier cytological and palynological analysis of three species of genus *solanum* at Saudi Arabia was done by Al-Wadi *et al.* (2007). The cytological study revealed that IPL-81 has highest value of mitotic index (M.I) i.e., 26.7% which revealed that this accession has highest power of division among present experimental accessions as compared to other accessions viz Sehore-74-3, DPL-15, 26.21% and 25.6% and lowest mitotic index in DPL-62 i.e., 9.52% indicated that this accession has lowest power of division(Table-1). To find out intervarietal correlation between cultivars, several earlier workers e.g., Jha and Ohri (1996), Ladizinsky (1979) made protein profiling study through SDS-PAGE and find almost same observations. Present investigation revealed that protein profiling is one of the basic and reliable methods to detect intervarietal genetic diversity and study phylogenetic relationship among the five selected experimental accessions of *Lens culinaris* (Table 1). During protein profiling of experimental accessions number of bands generated in different accessions of lentil. The protein band for highest molecular weight (i.e., 99 KD) in all accessions of lentil was generated in DPL-62 an lowest molecular weight (i.e., 40 KD) was generated in accession DPL-15.The bands observed in different accessions are twelve in IPL-406(L1), six in IPL-81(L2), ten in DPL-62(L3), eight in DPL-15(L4) and ten in Sehore-74-3 (L5). When bands of all accessions were compared, we obtained a total of 46 bands .Out of them 44 were polymorphic with 95.6% polymorphism. The near polymorphism percentage i.e., 100% was found in *Oryza sativa* L. Galani *et al*. (2010).Bhat and Kudesia (2011), found 100% in different five species of Solanaceae. This little change may be due to crop change. Jaccard's similarity coefficient ranged from 0.21429 to 0.83333.

The similarity index of five accessions of experimental crop was maximum between varieties IPL-406 (L1) , and Sehore-74-3 (L5) , i.e., 36.36% and next between DPL-15 (L4), and DPL-62 (L3) , i.e., 33.33% and then Sehore74-3(L5) and IPL-81(L2), DPL-62(L3) and IPL-81 (L2) i.e., 31.25%, while as similarity was lowest in IL-406 (L1), and DPL-15 (L4) , i.e., 15%. Dendrogram was constructed based on unweighted pair group method using arithmetic averages. Cluster analysis of data placed five accessions of lentil into two main clusters. Cluster first comprised one accession IPL-81(L2) and cluster first further subdivided into two subclusters comprising IPL-406(L1) and Sehore-74-3(L5).Cluster second comprised DPL-62(L3) , and DPL-15(L4).This is given in(Table-11,Figure-1).

Conclusion

Seed protein profiling through SDS-PAGE is the most important tool in determining the molecular polymphism and genetic-homology. Seed storage proteins helps in cultivars identification by avoiding the external environmental influences. During the present study of "Estimation of genetic diversity among different accession of lentil (*Lens cutlinaris*) by protein profiling and RAPD techniques" analyzed that the mitotic index was highest in IPL-81 (L_2) accession while as lowest was found in DPL-62 (L_3) which revealed that the accession IPL-81 has highest power of division while as DPL-62 has lowest. During present study 4 accession of lentil (i.e., IPL-406 (4) and Sehore-74-3; DPL-62 (L_3) with DPL-15 (L_4) show highest value of similarly index (i.e., 36.36%, and 33.33%) respectively which revealed that these accession are phylogenetically close to each other while as accession IPL-406 (L_1), DPL-15 (L_4) having similarly index (15%) which means that these accession are distantly related from each other. This has been proved at molecular level by using protein profiling among accession of lentil (*Lens cutlinaris*). Although these has been great advancement in the market technology with the advent of different DNA markers like Amplified fragment length polymorphism (AFLP), Simple Sequence repeats (SSR), Single Nucleotide Polymorphisms (SNPs) etc. Still RAPD is quite convenient to apply provided the problems of reproducibility is minimized. From the present investigation, it is clear that RAPD analysis revealed enough polymorphism in lentil. The only option left over is to validate by the samples provided. The preliminary work carried out with one random primer selected from literature revealing the genetic diversity between accession viz., IPL-51 (L_2), DDL-62 (L_3) and DPL-62 (L_3), Sehore-74-3 (4) age distantly related as per dendrogram constructed on the basis of UPGMA on the basis of RAPD data, could be exploited further by increasing the number of random primers and validating it with other available DNA markers. The accession which are distantly related either at protein or gene level should be used for plant breeding program in future.

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REFERENCES

Alabboud, Szilagyi L, Roman GV, 2009. Molecular research on the genetic diversity of lentil genotypes(*Lens culinaris*)using the RAPD method.*Lucrari Stiintifice*, 52: 753-757.

AL-wadi HM,Gamal MA, 2007. Palynological and cytological characters of three species of genus *solanum*(family *solanaceae*) from Saudi-Arabia.*Jour.of Bio*,4: 626-631.

Anwar R, Masood S, Khan MA, Nasim S,2003.The high molecular weight glutenin subunit composition of wheat landraces from Pakistan.*Pak.J.Bot*,35(1):61-68.

.Arumuganathan K, Earle ED, 1991. Nucleae DNA Content of some important plant species. *Plant Mol. Bio,* 9 : 208-218.

Bhat TM Kudesia R, 2011. Evaluation of genetic diversity in high different species of family *solanaceae* using cytological characters and protein profiling. *Genetic Engineering and Biotechnology Journal*, 20 :

Ertugruls Y, Sebahattin D, Leyla A, 2004. Analysis of genetic relationships among Turkish cultivars and breeding lines of *lens – culinaris mestile* using RAPD markers. *Genetic Resources and crop evolution*, 53 : 507-514.

Galani S, Naz F, Sromro F, Jamil I, Zia-ul-hassan, Azhar A, Ashraf A, 2010. Seed storage protein polymorphism in ten elite rice (*Oryza sativa L.*) genotypes of Sindh. *African Journal of Biotechnology*.,10(7) : 1106-1111.

Javaid A, Ghafoor A, Anwar R,2004.Seed storage protein electrophoresis in groundnut for evaluating genetic diversity.*Pak.J.Bot*,36:25-29.

Murphy RW, Sites JW, Buth DG, Haufler CH,1990.Protein1: Isozyme electrophoresis.*Mol.Syst*,45-126

Shibata D,2005.Genomic sequencing and functional genomics approaches in tomato.*Journal of General Plant Pathology*, 71:1-7.

Yu Z, Li L, Auan L, Die B, Man-Ye, Chen M, Ying-Fan C, Xia-Lin Q, Fang C, 2002. RAPD markers in diversity detection and variety identification of Tibet and Aullers barley. *Plant Mol. Biol. Reb*, 20: 369-377.

Welsh J, Mc Clelland M,1990.fingerprinting genomes using PCR with arbitrary primers. *Nucleic acids Res*,18:7213-7218.

Jha SS, Ohri D, 1996.Phylogenetic relationships of *Cajanus cajan* (L.) Millsp. (Pigeon pea) and its wild relatives based on seed protein profiles.*Grace,*43:275-281.

Ladizinsky,1979.The origin of lentil and its wild gene pool. *Euphytica,* 28:179-187.

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