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Genetic characterization of lentil genotypes based on SDS-PAGE

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Abstract
Lentil (*Lens culinaris* Medic) is a very important pulse crop. Fourteen Lentil genotypes namely; PL-4, KLS-218, KLS-320, L4147, K-75, KLB-08-4, KLS-09-3, VL-126, JL-1, L84-8, PL-5, KLB-303, IPL-81, DPL-62 were characterized on the basis of protein profiling through SDS-PAGE. Banding patterns through SDS-PAGE, found that the number of protein bands found in 14 genotypes ranged from 12 to 20 with Rm value 0.07 to 0.93 for Tris soluble proteins. UPGMA cluster analysis all the genotypes fall in seven cluster groups. SDS-PAGE for tris soluble proteins found suitable for testing distinctness, uniformity, stability of varieties for registration and identification. Further, characterization and identification of genotypes of lentil, on the basis of their protein electrophoretic profile through SDS-PAGE was resulted distinct banding pattern and act as 'genotypic finger printing'.

Keywords: lentil, protein profiling, SDS PAGE

1. Introduction
Lentil (*Lens culinaris* Medik.) is an important highly valued cool-season food crop of leguminous family (Liu and Muse, 2005) [7]. It is an important source of dietary protein (25 percent) in both human and animal diets, as a source of usable protein (CGIAR). Lentils contain high levels of proteins, including several essential amino acids like isoleucine and lysine an essential source of inexpensive protein in many parts of the world. Lentils are deficient in two essential amino acids, methionine and cystine. Lentil seeds are valued both as a food, high quality plant proteins and fiber and play an important role in crop rotations because their nitrogen fixing capability. Lentils are one of the best vegetable sources of iron. This makes them an important part of a vegetarian diet, and useful for preventing iron deficiency (Singh *et al.*, 2006) [16, 17]. Considerable variations among the characters had use in breeding and selection programmes have been reported and Many workers have been reported on genetic variation in lentil through morphological characters (Tullu *et al*., 2011) [21] and seed storage protein profile [Sultana *et al*., 2006] [20]. Proteins band were recorded with maximum relative mobility of 0.90 presence or absence of any particular band helps in demarcation and identification of variety. Lentil genotypes were distinguished based on presence and absence of protein bands at particular Rm value (Anuradha *et al*., 2010) [2]. Among biochemical techniques SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structures of crop germplasm (Javaid *et al*., 2004) [6]. Lentil is now mainly grown in Asia, but it is produced in the Americas where Canada is the leader. Global production of lentil is approximately 4.9 million tons which produces 1.8 million tons (FAO, 2013). Variations in environmental conditions stimulate plant breeders to develop lentil cultivars that are more resistant and tolerant (Sheikh *et al.*, 2011) [14]. There are several methods to study genetic diversity based on morphological, biochemical and molecular markers.
Genetic variation between and within populations of crop species is a major interest of plant breeders and geneticists because it facilities the efficient sampling and utilization of germplasm resource. The breeders must have the idea of choosing the accession that most likely possesses the trait of interest. The knowledge of genetic variation and relationships between populations is important to understand the available genetic variability and its utilization in breeding programs. Seed storage proteins profiling provides aid for identification and characterization of diversity in crop varieties and their wild varieties (Sammour, 1991) [13]. Seed storage protein markers can be used for selection and genetic diversity analysis (Yuzbasioglu *et al*., 2008; Sonmezoglu *et al*., 2010; Arslan, 2012) [18] of lentil. Keeping in view the above facts, the experiment was conducted on "Seed protein profiling through electrophoresis in lentil (*Lens culinaris* Medic) with objective to study the protein profiling of

lentil varieties and to analyze the similarity distance of clusters by tris soluble protein using UPGMA analysis.

Materials and Methods

The present investigation included 14 varieties of lentil (Table-1). Analysis of total soluble proteins for characterization of genotypes and total soluble proteins were analysed by SDS-PAGE using the method recommended by UPOV, 1994.

Table 1: Varieties under study for protein profiling.

S. No	Name of Variety	Source
1	PL-4	P.A.U,. Ludhiana
2	KLS-218	C.S.A.U.A&T., Kanpur
3	KLS-320	C.S.A.U.A&T., Kanpur
4	L 4147	P.A.U,. Ludhiana
5	K 75	C.S.A.U.A&T., Kanpur
6	KLB- 08- 4	C.S.A.U.A&T., Kanpur
7	KLS- 09-3	C.S.A.U.A&T., Kanpur
8	VL -126	U.P.I.C.A,. Almoda
9	JL – 1	JNKVU,. Jabalpur
10	L - 84 -8	PAU,. Ludhiana
11	PL – 5	G.B.P.U.A.&T., Pantnagar
12	KLB-303	C.S.A.U.A&T., Kanpur
13	IPL – 81	IIPR, Kanpur
14	DPL – 62	IIPR, Kanpur

SDS-PAGE method

Plant Sample

The lentil crop of 14 varieties was collected from Legume Section of the university. The analysis was carried out in Biotechnology Lab, Department of Genetics and Plant Breeding, C.S. Azad University of Agriculture and Technology, Kanpur.

SDS-PAGE analysis

The variability of seed storage-proteins was analyzed by using SDS-PAGE to investigate Seed protein profiling through electrophoresis in Lentil. The grains were ground to fine powder and 10 mg was weighed in 1.5 ml microtube,400 µl protein extraction buffer (Tris-Hcl 0.05M (pH 8), 0.02% SDS, 30.3% urea, 1% 2-mercaptoethanol) was added to each micro-tube, kept overnight at 40°C and centrifuged at 13000 rpm for 10 min. The supernatant contain dissolved extracted protein ready for experiment purposes, which could be kept for longer time at 4°C. The Preparation of Stock buffer for running gel (1.875M Tris-HCl buffers pH 8.8) was prepared by dissolving 22.69 g of Tris in 50 ml of distilled water. Then stacking gel (0.6 M Tris-HCl buffer pH 6.8) was prepared by dissolving 7.6 g of Tris in about 50 ml of distilled water and adjusted pH to 6.8 by adding conc. HCl drop by drop. The Stock SDS solution (10%) was prepared by dissolving 10 g SDS in distilled water with constant stirring and gentle heating. Stock protein extraction solution was prepared by dissolving 2g SDS and 10 mg methyl green in 10.4 ml 0.6 M Tris-HCl buffer (pH 6.6), 7.9 ml distilled water and 10 ml glycerol, warm gently and mixed well. Preparation of Ammonium persulphate (5%)was prepared by dissolving 0.5 g ammonium persulphate in distilled water to make 10 ml. to be prepared freshly just before use of Stock protein extraction solution was prepared by dissolving 2g SDS and 10 mg methyl green in 10.4 ml 0.6 M Tris-HCl buffer (pH 6.6), 7.9 ml distilled water and 10 ml glycerol and mixed well. Then Electrode (tank) buffer was prepared by dissolving 9.0 g Tris, 42.3 g glycine and 3 g SDS in distilled water by adjusting pH of buffer was 8.3. Staining solution was prepared by dissolving 100 ml of 15% TCA solution, add 10 ml of 1% commassie brilliant blue prepared in methanol (*i.e.* 1g comassie brilliant blue dissolved in 100 ml methanol).The defatting solvent mixture(2:1:1-chloroform:acetone:methanol) solution was prepared by mixing 200 ml of chloroform, 100 ml methanol and 100 ml acetone.

Preparations of stacking and running gel

It was prepared by dissolving 75g acrylamide and 1g bisacrylamide in distilled water to make 250 ml whereas stacking gel was prepared by dissolving 75g acrylamide and 2g bisacrylamide in distilled water to make 250 ml.

Protein Extraction

About 1 g seed were grinded in mortar and pestle after removing the seed coat and defatted by defatting solution 4 times. 1 ml Tris-glysine extraction buffer (pH 8.3) was added to 0.5g of defatted powder and left over night. 10 % solution of SDS (10µl), 2-mercapto ethanol (10µl) with bromophenol blue (10 µl) was added. Mixed well and left over night in a refrigerator. The mixture was kept for 10 minutes in water bath at 100ºC. The mixture was centrifuged at 10,000 rpm for 15 minutes at 4ºC. Clean suspension supernatant was used for study. Electrophoresis was conducted using Atto Electrophoresis Unit having fourteen well for loading the sample fixed the gel cassette into the electrophoresis unit, as per the design of the equipment. loaded 50 µl of clear supernatant to each well. Conducted electrophoresis at 42 mA (@ 1.5 mA per well) till the sample migrates into the running gel, and subsequently at 56 mA unit the tracking dye reaches the bottom of the gel. The cassette was removed from the unit and take out the gel gently. Place it in a staining tray and incubate overnight in 15% TCA (Trichloroacetic acid) solution. Washed thoroughly the excess SDS which might precipitate on the surface sufficient staining solution to cover the gel uniformly incubation for 16 hr to stain and rinsed with water. Destaining in water and 5% Acetic acid for a day or two till clears the gel background, resulting in a better resolution. Place the gel over a trans-illuminator and draw the electrophoregram for calculating Rm values. Photograph of the gel was also being taken. Analysis of total soluble proteins for characterization of genotypes and total soluble proteins were analysed by SDS-PAGE using the method recommended by UPOV, 1994.

Results

Results and Discussion

The crop improvement programmes, aims to identify suitable genotypes and develop varieties with better agronomic characters, resistance against biotic and abiotic stresses and better quality parameters. Hence, characterization of genotypes, was importance to establish the identification key and also to protect the Breeder's Right for its commercial use. Morphological descriptions are useful in assessing the genetic purity and identity the varieties. However, these techniques are being time consuming, laborious and also being influenced by environment. Therefore, it is needed to develop rapid reliable and reproducible techniques for distinguishing varieties.Biochemical marker can be used for distinguishing varieties because they are stable, reproducible and can be conducted relatively in short time.

Characterization based on protein profiling through SDS-PAGE

The protein band patterns obtained from the 14 lentil cultivars are shown in Figure 1.Only clearly distinguishable protein bands were visually scored. A total of 23 polypeptide bands were detected and molecular weight ranged from 10 to 200 kDa. Erdogan (2015) [3] reported that band patterns obtained from the 13 lentil cultivars and total of 22 polypeptide bands were detected. Presence and absence of bands, which are identified by their respective, relative mobilities and number in sequence from cathodal origin. Bands, which are common in genotypes according to bands and their Rm value. The number of bands present in 14 genotypes ranged from 12 to 20 with Rm value 0.07 to 0.93. The minimum 12 band were observed in KLB -303; 13 bands present in L- 4147, KLB 08-4, KLS-09-3, L- 84-8, PL-5, DPL- 62; 14 bands present in KLS- 320, VL-126, JL- 1; 15 bands present in KLS-218, K-75; 17 bands present in PL- 4, and maximum 20 bands were observed in IPL-81.Based on the Rm value total of 23 protein bands were identified at different Rm value. Bands having Rm value 0.29 and 0.45 are common in all varieties.

This method provides powerful tool for variety discrimination and identification based on genetic differences in seed storage protein composition of genotypes of lentil. Genotypes were distinguished based on presence and absence of protein bands at particular Rm value and total numbers of bands present. Bands, which showed similarity and dissimilarity among genotypes on the basis of bands present or absent and their Rm value (Table: 1, 2).Bands with Rm value 0.29 and 0.45 were present in all the genotypes. Similarity matrices, estimated from simple matching coefficients, were used for building a dendrogram by the UPGMA method with 22 SDS-PAGE bands (Fig.4). Genetic similarity coefficients varied among lentil cultivars (Table 1). The dendrogram (Figure 2) clearly revealed three separate main clusters at the 0.75 similarity coefficient level. Clusters I contain three genotype in grouped namely PL-4, KLS-326 and K-75, in which KLS-320 and K-75 are more close than PL-4.Where as cluster, II and III KLB 08-4 and DPL-62, respectively have wider distance to other genotypes. In Cluster IV, KLS-218 and VL-126 are grouped that are contain two genotype which are close to each other. Cluster V contains two genotype L 4147 and JL-1 are grouped that are close to each other. Cluster VI contains three genotype L84-8, PL-5 and KLB-303 are grouped in which PL-5 and KLB-303 are more close than L 84-8. Cluster VII contain two genotypes KLS 09-3 and IPL-81 that are close to each other.

Lentil is an important food legume known for its quality. Therefore, improving new lentil cultivars is essential for sustainable production. We need the revelation of genetic variation among lentil genotypes to breed a new variety with the desired agronomic and commercial characters. As a result of SDS-PAGE analysis, variations in seed protein patterns were observed among the lentil cultivars. There was a high degree of homogeneity in the six major protein bands (Figure 1). Uniformity in the major bands among various cultivars indicates that these proteins are conserved by gene coding (Javaid *et al*., 2004) [6]. However, there were variations in the minor bands. The similarity matrices of SDS-PAGE (Table 1) indicated higher genetic similarity estimates among lentil cultivars than by although the overall estimates of genetic similarities in both analyses were high. This may be because plant breeders narrow genetic diversity in their breeding populations by selecting the required trait combinations for outputs of improved cultivars (Redden *et al*., 2007) and suggest the similarity of genes responsible for seed storage proteins (Ali *et al*., 2007; El-Nahas *et al*. 2011) [1, 11]. SDSPAGE the protein markers, is an easy, safe, and efficient method to discriminate lentil cultivars (Hoque and Hasan, 2012) [5].

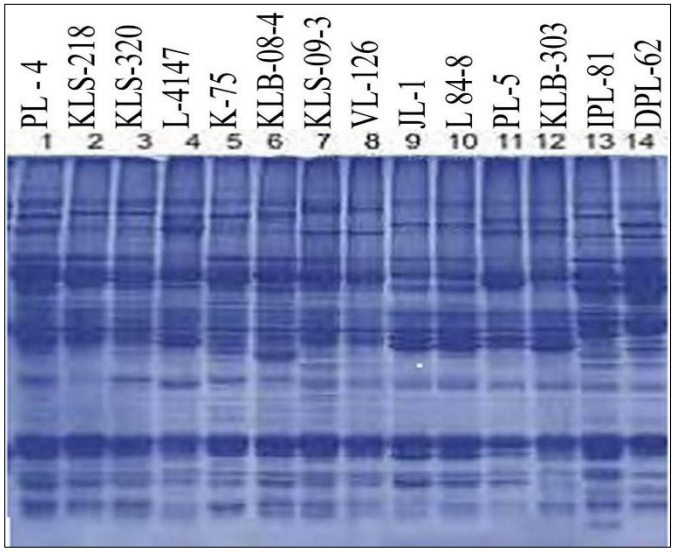


Fig 1: SDS-PAGE Electrophoregram of Tris Soluble Proteins Lentil genotypes

Table1: Relative mobility Rm values and presence/absence of total soluble seed protein in Lentil genotype (SDS-PAGE)

Band No.	Genotype Rm value	PL-4 (1)	KLS 218 (2)	KLS 320 (3)	L 4147 (4)	K 75 (5)	KLB 08-4 (6)	KLS 09-3 (7)	VL 126 (8)	JL 1 (9)	L 84-8 (10)	PL 5 (11)	KLB 303 (12)	IPL81 (13)	DPL 62 (14)
1.	0.07	+	-	-	+	-	-	+	-	-	-	-	-	+	+
2.	0.11	-	-	+	+	+	+	-	-	+	-	-	-	-	-
3.	0.12	+	+	-	-	-	-	+	+	-	+	+	+	+	+
4.	0.14	+	+	+	+	+	+	-	-	-	-	+	+	+	-
5.	0.18	+	+	+	+	+	+	-	+	+	+	+	+	+	-
6.	0.25	+	+	+	-	+	+	+	+	+	+	+	+	+	+
7.	0.29	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.	0.33	+	+	+	+	-	+	-	+	+	+	+	+	+	+
9.	0.37	+	+	+	-	+	+	-	+	-	-	-	-	-	+
10.	0.39	+	+	+	-	+	-	+	-	+	+	+	+	+	-
11.	0.43	+	-	-	-	+	+	+	-	-	-	-	+	+	+
12.	0.45	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13.	0.48	-	-	-	+	-	-	-	-	+	+	+	+	+	-
14.	0.50	+	-	+	-	+	+	-	-	-	+	-	-	+	+

15.	0.53	-	-	-	-	-	-	+	+	-	+	-	-	+	+
16.	0.58	+	+	-	+	+	+	+	+	+	-	+	+	+	+
17.	0.61	+	+	+	+	+	-	+	+	+	--	-	-	+	-
18.	0.71	-	+	-	-	-	-	+	+	+	+	+	+	+	-
19.	0.74	+	+	+	+	+	+	+	+	+	-	-	-	+	+
20.	0.82	+	+	+	+	+	-	-	+	+	+	+	-	-	+
21.	0.86	-	-	-	-	-	-	-	-	-	-	-	-	+	-
22.	0.88	+	+	+	+	+	+	+	+	+	+	+	-	+	+
23.	0.93	-	-	-	-	-	-		-	-	-	-	-	+	-
	Total	17	15	14	13	15	13	13	14	14	13	13	12	20	13

Table 2: Electrophoretic profile of tris soluble protein through SDS-PAGE :

S. No	Genotype	Rm values of bands present
1	PL-4	RM Value 0.07, 0.12, 0.14, 0.18, 0.25, 0.29, 0.33, 0.37, 0.39, 0.43, 0.45, 0.50, 0.58, 0.74, 0.82, 0.88 present
2	KLS-218	Rm Value 0.12, 0.14, 0.18, 0.25, 0.29, 0.33, 0.37, 0.39, 0.43, 0.45, 0.58, 0.71, 0.74, 0.82, 0.88 present.
3	KLS-320	Rm Value 0.11, 0.12, 0.18, 0.25., 0.29, 0.33, 0.37, 0.45, 0.50, 0.74, 0.82, 0.88 present
4	L 4147	Rm Value 0.07, 0.11, 0.12, 0.18, 0.29, 0.33, 0.45, 0.48, 0.58, 0.74, 0.82, 0.88 present
5	K-75	Rm Value 0.11, 0.12, 0.18, 0.25, 0.29, 0.37, 0.39, 0.43, 0.45, 0.50, 0.58, 0.61, 0.74, 0.82, 0.88 present
6	KLB-08-4	Rm Value 0.11, 0.14, 0.18, 0.25, 0.29, 0.33, 0.37, 0.43, 0.45, 0.50, 0.58, 0.74, 0.88 present
7	KLS -09-3	Rm Value 0.07, 0.12, 0.25, 0.29, 0.39, 0.45, 0.45, 0.53, 0.58, 0.61, 0.71, 0.74, 0.88 present
8	VL-126	Rm Value 0.12, 0.18, 0.25, 0.29, 0.33, 0.37, 0.45, 0.53, 0.58, 0.61, 0.71, 0.74, 0.82, 0.88 present
9	JL-1	Rm Value 0.11, 0.18, 0.25, 0.29, 0.33, 0.39, 0.45, 0.478, 0.58, 0.61, 0.71, 0.74, 0.82, 0.88 present
10	L-84-8	Rm Value, 0.12, 0.18, 0.25, 0.29, 0.33, 0.39, 0.45, 0.48, 0.50, 0.53, 0.71, 0.82, 0.88 present
11	PL-5	Rm Value 0.12, 0.14 0.18, 0.25, 0.29, 0.33, 0.39, 0.45,0.48, 0.58, 0.71, 0.82, 0.88 present
12	KLB-303	Rm Value 0.12, 0.14 0.18, 0.25, 0.29, 0.33, 0.39,0.43, 0.45, 0.48, 0.58, 0.71, 0.88 present
13	IPL-81	Rm Value 0.07, 0.12, 0.14, 0.25, 0.29, 0.33, 0.39, 0.43, 0.45, 0.48, 0.51, 0.53, 0.58, 0.61, 0.71, 0.74, 0.86, 0.93 present
14	DPL-62	Rm Value 0.07, 0.12, 0.18, 0.25, 0.29, 0.33, 0.37, 0.43, 0.45, 0.50, 0.53, 0.58, 0.74, 0.82, 0.88 present.

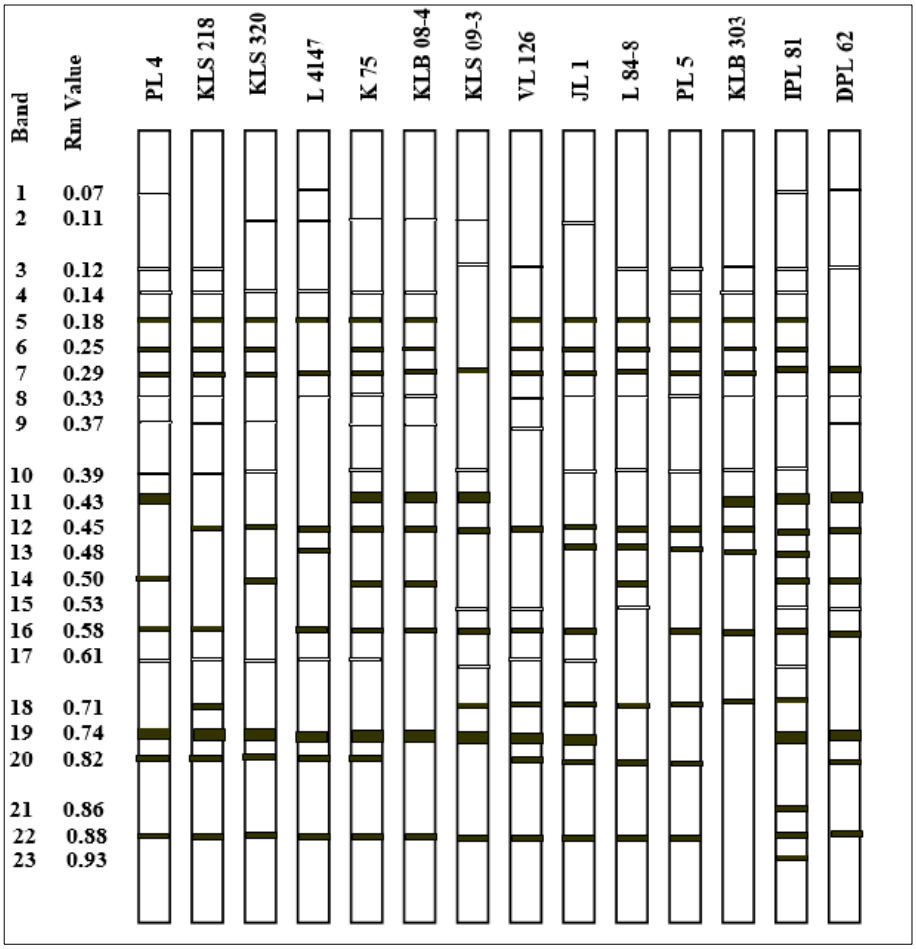
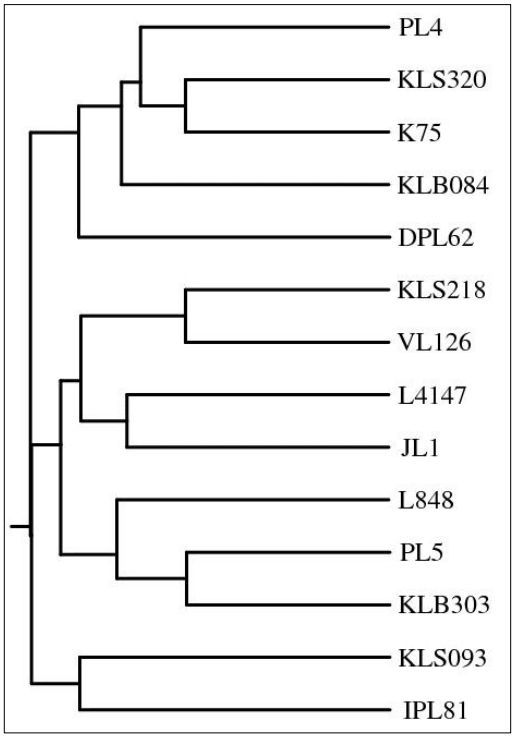


Fig 2: Electrophoresis of Lentil varieties showing protein banding pattern through SDS-PAGE



SDS-PAGE Dendrogram of 14 varieties of lentil seed based on protein banding pattern using UPGMA cluster analysis

Conclusions

On the basis of results, this can be said for characterization and identification of genotypes of lentil, that electrophoretic profile for tris soluble proteins through SDS-PAGE was resulted distinct banding pattern and act as 'genotypic finger printing'. Therefore, electrophoregram of tris soluble protein in SDS-PAGE was found much better for identification of genotypes in lentil.

References

1. Ali Z, Qureshi AS, Ali W, Gulzar H, Nisar M, Ghafoor A. Evaluation of genetic diversity present in pea (Pisum sativum L.) germplasm based on morphological traits, resistance to powdery mildew and molecular characteristics. Pakistan Journal of Botany. 2007; 39:2739-2747.
2. Anuradha Singh, Mohammad Shahid, Vyas RP. Genotypic identification of lentil (*Lens culinaris)* using electrophoresis technique. Trends of Biosciences. 2010; 3(2):225-227.
3. Erdogan C. Genetic characterization and cotyledon color in lentil. Chilean journal of agricultural research. 2015; 75(4). S0718-58392015000500001.
4. Ghafoor A, Arshad M. Seed protein profiling of Pisum sativum (L) germplasm using Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for investigation of biodiversity. Pakistan Journal of Botany. 2008; 40(6):2315-2321.
5. Hoque ME, Hasan MM. Molecular diversity analysis of lentil (Lens culinaris Medik.) through RAPD Markers. Plant Tissue Culture and Biotechnology. 2012; 22:51-58.
6. Javaid A, Ghafoor A, Anwar R. Seed storage proteın electrophoresıs in groundnut for evaluating genetic diversity. Pakistan Journal of Botany. 2004; 36(1):25-29.
7. Liu K, Muse SV. Power Marker: Integrated analysis environment for genetic marker data. Bioinformatics. 2005; 21:2128-2129.
8. Mandaokar AD, Koundal KR, Kansal Rekha, Bansal HC. Characterization of vicilin seed storage protein of chickpea (*Cicer arietinum* L.). Journal of Plant Biochemistry and Biotechnology. 1992; 2(1):971-7811.
9. Mandaokar AD, Koundal KR, Kansal Rekha, Bansal HC. Characterization of vicilin seed storage protein of chickpea (Cicer arietinum L.). Journal of Plant Biochemistry and Biotechnology. 1992; 2(1):971-7811.
10. Mckee CW. Chemical and biochemical technique for varietal identification. Seed Sci. and Technol. 1973; 1:181-191.
11. ElNahas AI, ElShazly HH, Ahmed SM, Omran AAA. Molecular and biochemical markers in some lentil (Lens culinaris Medik.) genotypes. Annals of Agricultural Sciences. 2011; 56:105-112.
12. Piergiovanni AR, Taranto G. Assessment of the genetic variation in Italian lentil population by electrophoresis (SDS-PAGE) of seed storage proteins. Plant Genetic Resources Newsletter. 2005; 141:33-38, 31.
13. Sammour RH. Using electrophoretic techniques in varietal identification, biosystematic analysis, phylogenetic relations and genetic resources management. Journal of Islamic Academy Science. 1991; 4(3):221-226.
14. Sheikh GN, Ahmad S, Kudesia R. Estimation of Genetic Diversity in Lentil (Lens Culinaris) Using Protein Profiling and RAPD. International Journal of Current Research. 2011; 3(12):017-021.
15. Singh AK, Kumar P, Singh J, Rani R, Rani A, Shukla P *et al*. Biochemical Profiling of Lentil (Lens Culinaris Medik) Germplasm at Different Growth Stages. Journal of biological engineering research and review. 2014; 1(2):01-06.
16. Singh K, Khare D, Rao S. Identification of lentil by seed protein electrophoresis. Seed Research. 2006; 34(1):40-44.
17. Singh, Kanchani, Khare D, Rao S. Identification of lentil by seed protein electrophoresis. Seed Research. 2006; 34(1):40-44.
18. Sonmezoglu OA, Yildirim A, Gulec TE, Kandemir N. Markor destekli seleksiyonun bugday islahinda kullanimi. Journal of Agricultural Faculty Gaziosmanpasa University. 2010; 27:105-112.
19. Sultana T, Ghafoor A. Genetic diversity in ex- situ conserved Lens culinaris for botanical descriptors, biochemical and molecular markers and identification of landraces from Indigenous Genet Resource. Pakistan of Journal Integration of Plant Biology. 2008; 50:484-490.
20. Sultana T, Ghafoor A, Ashraf M. Geographic pattern of diversity of cultivated lentil germplasm collected from Pakistanas assessed by protein assays Acta Biologica Cracoviensia, Series Botanica. 2006; 48:77-84.
21. Tullu A, Diederichsen A, Suvorova G, Vandenberg A. Genetic and genomic resources of lentil: status, use and prospects. Plant Genetic Resources. 2011; 9(1):19-29.
22. Yuzbasioglu E, Ozcan S, Acik L. Analysis of genetic relationships among Turkish cultivars and breeding lines of *Lens culinaris* Mestile using RAPD markers. Genetic Resources and Crop Evolution. 2006; 53:507-514.