SEED PROTEIN PROFILING THROUGH ELECTROPHORESIS IN LENTIL [*LENS CULINARIS* MEDIC]

Prafull Kumar[1], Avadhesh Kumar Koshal[2] , Sanjay Kumar[3] and Manoj Kumar Sharma[3]

[1]Department of Genetics and Plant breeding, C.S. Azad University of Agriculture and Technology, Kanpur-208 002, (Uttar Pradesh) India
[2]Project Directorate for Farming Systems Research (P.D.F.S.R.), Modipuram, Meerut (Uttar Pradesh) India
[3] Sanjay Kumar, Janta Vedic College, Baraut, Baghpat (Uttar Pradesh) India

Abstract: Lentil (*Lens culinaris* Medic) is an important pulse crop in India with advancement and development of hundreds of varieties and introduction of intellectual property rights it is necessary to identifying them individually for identification and registration purposes. The present investigation was carried out during 2012-2013 in biotechnology lab, Department of genetics & Plant Breeding, C.S. Azad university of agriculture and technology, Kanpur with 14 genotypes of Lentil 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 for protein profiling through SDS-PAGE.

In present investigation, 14 variety of Lentil were studied for varietal identification through electrophoresis. Protein was extracted from dry seed of lentil varieties and analysed by SDS-PAGE . On the basis of photographs, electrophoregrams, Rm values and dendograms (UPGMA cluster analysis) of banding patterns through SDS-PAGE, results 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. Protein banding pattern of tris soluble proteins was found more distinct in SDS-PAGE. In 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.

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.

Keywords: Lentil, SDS-PAGE, Varietal identification, UPGMA

INTRODUCTION

Lentil *(Lens culinaris* Medic) is the second most important winter pulse crop in India. About half of the world production of lentils in from India. The important lentil growing countries are India, Turkey, Syria and Bangladesh, the lentil crop having high nutritive value 100 g of lentil contain 26 g protien. most of which is consumed in the domestic market with the advancement and development of thousands of improved new varieties and introduction of IPR, it is necessary to identifying them individually for identifications and registration purposes. For the purpose necessity of quick, reliable and reproducible laboratory techniques are required. Protein profiling through SDS-PAGE is an alternate techniques for distinguishing the genotypes. Protein markers are stable, reproducible and genetically controlled and can be conducted in relatively short time.the protein profiling of seed storage proteins in cultivated lentil and their significantly differences in banding patterns by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

In electrophoresis, substances in a mixture, which are ionisable, can be reported from others that are net, by subjecting the mixture to an electric field: SDS-PAGE is the most commonly methods used for studying protein differences between species, previously classified on the basis of their morphological characters. SDS is an anionic detergent which binds strongly and denatures proteins. The number of SDS molecule bound to a polypeptide chain is approximately half the no. of amino acid residues in that chain. The protein SDS complex carries net negative charges. Hence, more towards the anode and the separation is based on the size of the protein.

MATERIAL AND METHDOS

The study was conducted during 2012-13 at Biotechnology Lab, Department of Genetics and Plant breeding, C.S. Azad University of Agriculture and Technology, Kanpur (UP). For the study, genetically pure seeds of 14 lentil genotypes viz Lentil 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 procured from the lentil Breeder of the university on the basis of morphological characters. The soluble proteins were analysed through SDS-PAGE method recommended by Dadlani *et al.*, 1993 for variety identification

Preparation of Sample

About 1 g seed were grinded in mortar and pestle after removing the seed coat and defatted by defatting solution 4 times of all 14 genotypes were taken in tube separately 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 sample was heated in boiling water bath kept for 10 minutes in water bath for 10 minutes at 100ºC. . The tubes were cooled and centrifuged at 10000 rpm for 10 minutes. The clear supernatant was used for electrophoresis.

Preparation of gel

Seed protein were analysed through slab type SDS-PAGE followed by Laemmli (1970) using 12% polyacrylamide gel. Electrophoresis was conducted in Atto Electrophoresis Unit using fourteen well for loading the sample.fixed the gel cassette into the electrophoresis unit as per design of the equipment. Loaded 50 µl of clear supernatant with one drop of tracking dye (Bromophenol blue) was loaded to each well. The electrophoresis was conducted at a constant current of 1.5 mA per well [42 mA] till the tracking dye crossed the stacking gel than current was fixed @ 2 mA per well at 220 V. The electrophoresis was stopped after the tracking dye reached the bottom of the gel.

Fixing and staining

Removed the cassette from the unit and take out the gel gently. Placed it in a staining tray and incubate overnight in 15% Trichloroacetic acid solution. Wash thoroughly the excess SDS, which might precipitate on the surface. Sufficient 1% commassie brilliant blue solution, prepared in methanol was added to cover the gel uniformly and incubated for 16 hr to stain than rinsed with water. Destaining in water and 5% Acetic acid for two days clears the gel background, resulting in a better resolution. The gel was placed over a trans-illuminator to draw the electrophoregram for calculating Rm values.

RESULT AND DISCUSSION

Results obtained through SDS-PAGE showed that the method provided a powerful tool for reliable variety discrimination and identification based on genetic differences of seed storage protein composition. Genotypes were distinguished on the basis of presence and absence of protein bands at particular Rm value and total numbers of bands present.

In the electrophoregram of tris soluble protein through SDS-PAGE (Fig. 1 & 2) in 14 genotypes, the number of protein bands were ranged from 12 to 20 with Rm value 0.07 to 0.93.

The lentil genotypes based on similarity distance dendrogram of 14 genotype of tris soluble protein banding pattern using UPGMA clusters analysis (Fig. 3) were grouped in 7 clusters. Clusters first contain three genotypes in grouped namely ; PL-4, KLS-326 and K-75, in which KLS-320 and K-75 are more close than PL-4.cluster, second and third KLB 08-4 and DPL-62, respectively have wider distance to other genotypes.Cluster fourth, KLS-218 and VL-126 are grouped that are contain two genotypes which are close to each other.Cluster fifth contains two genotypes L 4147 and JL-1 are grouped that are close to each other.Cluster six contains three genotypes L84-8, PL-5 and KLB-303 are grouped in which PL-5 and KLB-303 are more close than L 84-8.Cluster seven contain two genotypes KLS 09-3 and IPL-81 that are close to each other.

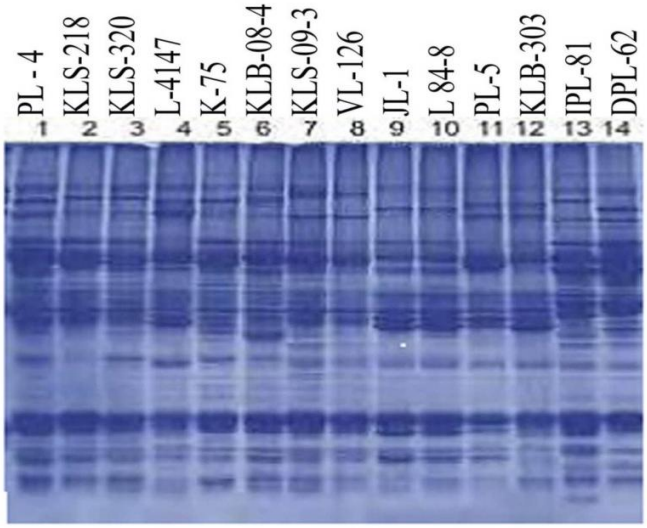

Fig1:SDS-PAGE Electrophoregrams of Tris Soluble Proteins in Lentil genotypes

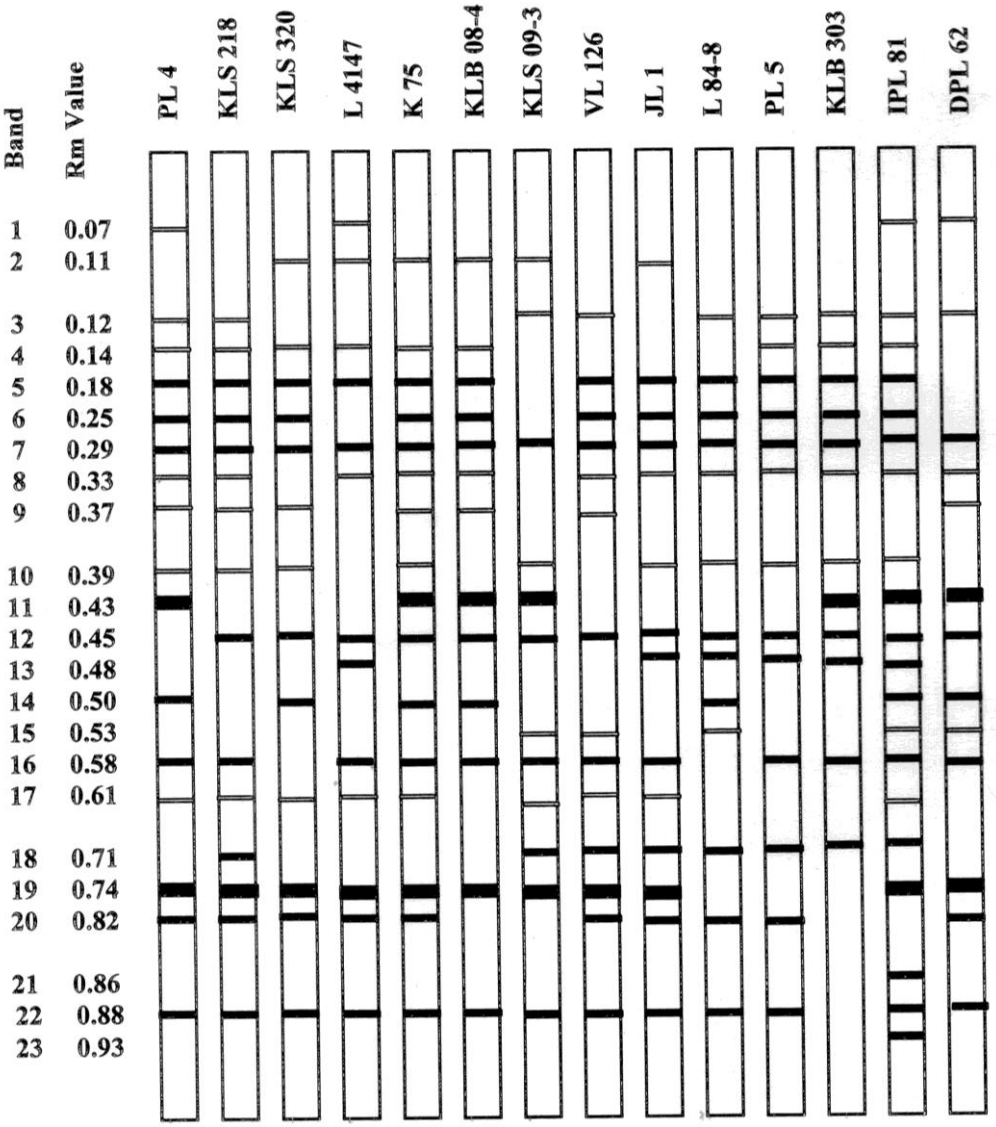

Fig. 2: Electrophoresis of Lentil varieties showing protein banding pat through SDS-PAGE

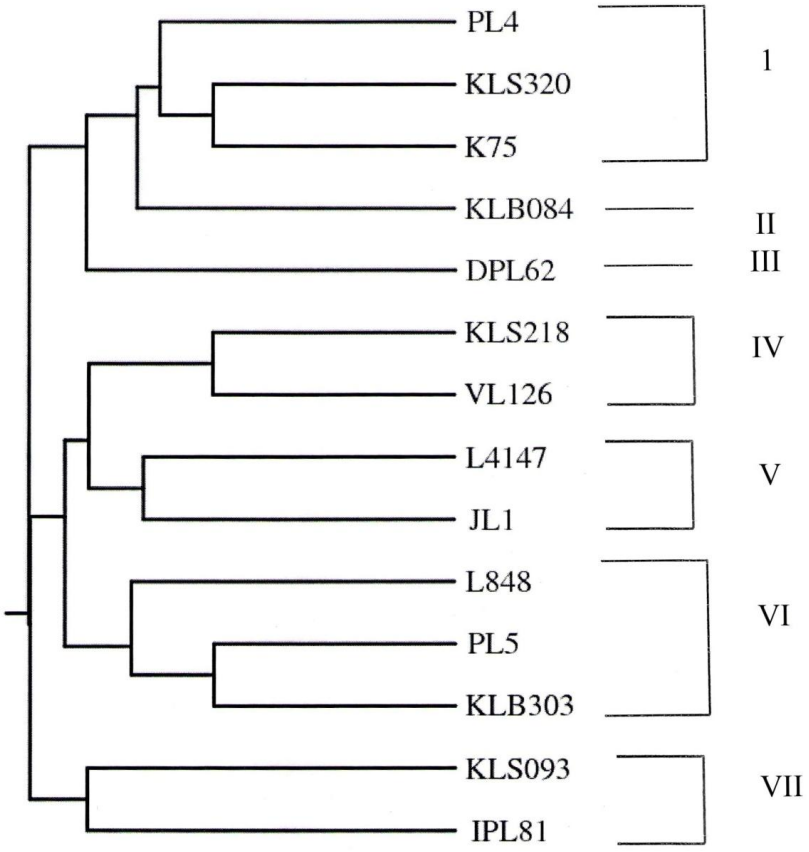

Fig. 3: SDS-PAGE Dendrogram of 14 varieties of lentil seed based on protein banding pattern using UPGMA cluster analysis

On the basis of photographs, electrophoregrams, Rm values and dendograms (UPGMA cluster analysis) of banding patterns through SDS-PAGE, variations were found in Rm value of protein bands, numbers of protein bands, similarity distance cluster analysis. These results are supported to the findings of and Singh *et al.,* (2006). Anuradha *et al.,* (2012) Protein banding pattern of tris soluble proteins were found more distinct. 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 much better.

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