PHYLOGENETIC RELATIONSHIP OF LENTIL SEED STORAGE PROTEINS WHICH BELONGS TO *Lensculinaris.*

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***Abstract*- Genetic diversity analysis of thirty-two lentil genotypes was performed using sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) electrophoresis based on their total seed storage protein. These genotypes were divided into ten distinct clusters based on their electrophoretic patterns using a dendrogram created using the genetic similarity coefficient matrix based on SDS-PAGE. The thirty-two genotypes examined here are from lentil species. All 32 of the genotypes in this group are members of the *Lens culinaris* genus. With the electrophoretic profiling of the different lentil genotypes, the pedigree analysis of genotypes shows similar parentage. From this study, it has been concluded that the total seed storage protein profile of lentil can be used as a marker for genetic diversity.**

***Keywords*: Seed storage proteins, genetic diversity, *Lens culinaris*, SDS-PAGE, dendogram**

1. Introduction

Lens is a self-pollinating, diploid (2n – 2x = 14), cool-season legume with a huge genome size of approximately 4 Gbp (Singh et al, 2018). Cultivated lentil comes in third position after chickpea and pea in terms of worldwide production1. Lentils were first domesticated in the Eastern Mediterranean during the Neolithic era, between 7000 and 10,000 years ago (Gupta et al, 2011). It is native to southwest Asia and the Mediterranean, lentil and its wild species are now grown in North and South America, Australia, and other parts of the world (Hamwieh et al, 2009). More than 58 countries produce lentils, along with Canada producing 48.1% of the worlds supply and accounting for 64% of exports worldwide. India continues to be the worlds top importer of lentils despite being the second-largest producer (Agarwal et al, 2008; Chahota et al, 2019).

Among legumes, lentil (*Lens culinaris*) contains a high amount of protein (22-26% dry weight; Longobardi et al, 2017). According to studies by Ibanez et al. (2003) and Longobardi et al. (2017), lentil is also a good source of vitamins, dietary fiber, and minerals. In comparison to other legumes like chickpeas, cowpeas, lupins, faba beans, and pigeon beans, lentil has a lower fat content and trans-fatty acid content (Ene-Obong and Carnovale, 1992; Fao, 2019; Urbano et al, 2007). Over 5.7 million tons of lentils were produced worldwide in 2019, with the top producers being China, India, Nepal, Bangladesh, Canada, and the United States (Fao, 2019; Rawal and Navarro, 2019).

Seed storage proteins have potential applications as markers in plant breeding, genetic diversity research, a key tool for crop improvement, and genotype classification (Ghafoor and Ahmad, 2005). These genetic markers have several drawbacks, hence a more effective strategy should be used to identify genotypes using protein markers, such as seed storage proteins. For protein-based classification, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) appears to be the most successful method because it is fast, easily affordable, and mostly unaffected by the growth environment (Sammour, 1991). As seed storage proteins do not denature during seed production, they are more stable than other proteins (Jin et al, 2006). SDS-PAGE has quickly developed into a useful technique for analyzing genetic diversity (Fazal et al, 2012). SDS-PAGE is a reliable approach for seed storage protein analysis since it is not affected by environmental factors (Anu and Peter, 2003). Because they are typically unaffected by environmental fluctuations, seed storage proteins and nucleotide sequences are particularly helpful and reliable approaches (Iqbal et al, 2005). The seed storage proteins research has been reported13 in numerous investigations such as phylogenetic interactions, genetic diversity, and genomic homologies. Protein characterization has also been utilized to decipher the genetic links between a collection of cultivars. Genetic diversity analysis of lentil hasn't been explored before based on seed storage protein profile, notably for lentil genotypes. As a result, based on the profiling of seed storage proteins, relatively little information is published on the evolutionary link between the numerous lentil types. Based on the polypeptide pattern of the total seed protein, the evolutionary relationship between 32 genotypes of lentil that correspond to the *Lens culinaris* species was determined.

2. Materials and Methods

2.1 Plant Material

Lentil genotypes used in this study were procured from CCS Haryana Agricultural University, Hisar, Haryana, Punjab Agricultural University, Ludhiana, Punjab and National Bureau of Plant Genetic Resources, New Delhi.

2.2 Seed Protein Extraction

Singh and Matta (2011), method was used for the preparation of total seed protein extracts by mixing the defatted seed meal in Tris-HCl buffer solution (0.2 mol ·L− 1, pH 6.8) containing 2% SDS. Twenty mg of seed meal was suspended in 400 µL buffer solution heated at 80 °C in a water bath for 45 min. The contents were centrifuged at 10,000 rpm for 10 min and supernatant used for

analysis. Glycerol was added to the sample meal containing the extracts so that it amounted to 10% of the final volume. To run the proteins under reducing conditions, 2-mercaptoethanol was added to limit its concentration to 2% in the total protein extracts.

2.3 SDS-Polyacrylamide Gel Electrophoresis

SDS polyacrylamide gel electrophoresis was prepared according to the formulation of Laemmle (1970). The total protein extracts were analysed in the discontinuous system of gel electrophoresis. The 1.5 mm thick gel was cast in the glass plates forming slab gel molds. The lower separation gel was prepared by using the stock solution

2.4 Phylogenetic Relationship

The presence (1) and absence (0) of the protein bands on the SDS-PAGE is used for the genetic diversity analysis of the polypeptide patterns of all the lentil genotypes. Phylogenetic relationship between all the lentil genotypes was carried out using SPSS (Version 28.0) software in the form of unweighted pair group method with arithmetic average (UPGMA) dendrogram as described by Singh and Matta.

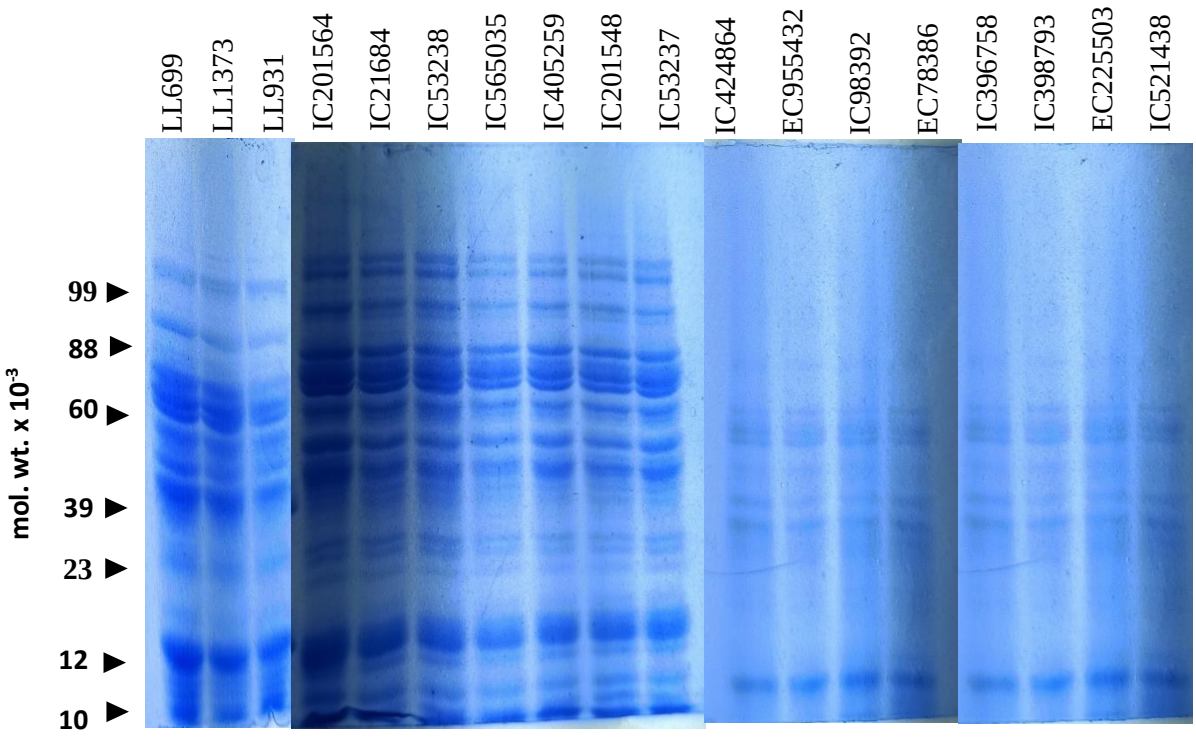
2.5 Statistical Analysis

The SPSS Version 28 was used for the data analysis

3. Results

3.1 Electrophoretic Variation in Lentil Genotypes (gels)

The polypeptide pattern of the total seed storage protein of the thirty-two lentil genotypes was performed to study the genetic diversity among these genotypes on the SDS-polyacrylamide gels under reducing conditions. The phylogenetic relationship was drawn between the lentil genotypes. The four genotypes of *Lens culinaris* showed similarity in 16 polypeptides on SDS-PAGE and only differences were recorded in IC98392, IC614705 at the position of mol. wt. 99kDa, 32 kDa. Similarly, the seed protein extracts of IC396758, EC225503 showed similarity in 14 polypeptides with IC201564 and differences recorded at the position of mol. wt. 60 kDa, 33kDa and placed in cluster II. In cluster-III EC955432, EC78386, IC53237 are similar with each other and IC405259, IC201684 having differences in their polypeptide pattern at the position of mol. wt. 91 kDa, 71 kDa with IC201684, IC95654. IC53238 doesn't showed similarity with other genotypes hence placed singly in cluster-VI. Based on electrophoretic patterns genotypes LH84-8, LH82-6 are similar with each other. LL699, EC28514 of cluster VII having difference only at mol. wt. 53 kDa, 13 kDa. LH89-98, IC201693, LL1373, LL931 these four lentil genotypes revealed the different polypeptide pattern of total seed protein extracts and therefore placed singly in four different clusters.



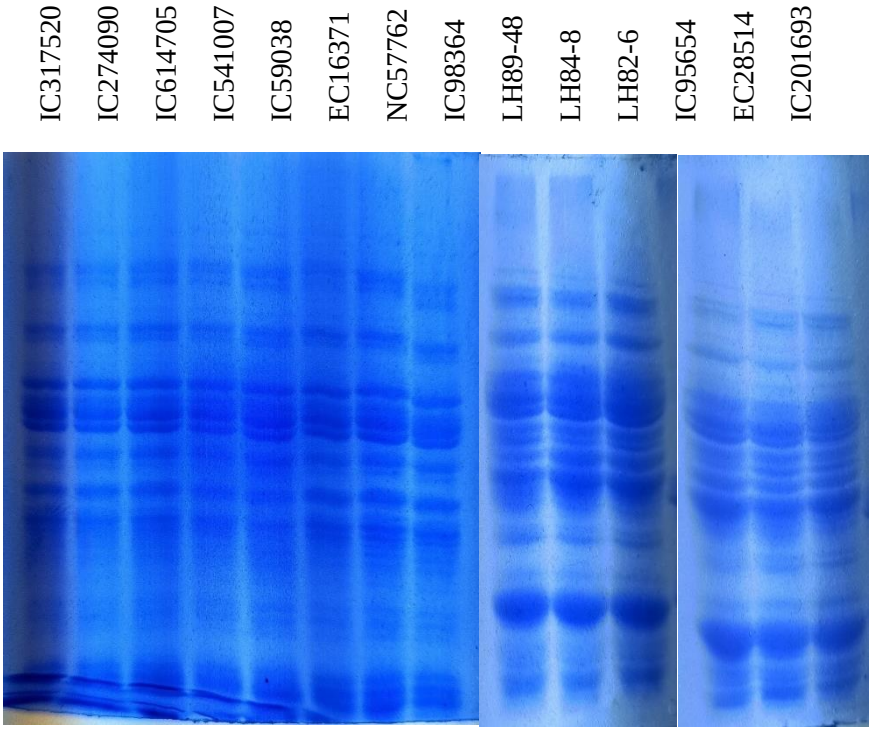


Fig 1: SDS-polyacrylamide gel electrophoresis of seed protein extracts of *Lens culinaris*

3.2 Phylogenetic Analysis of Lentil Genotypes

The genetic similarity coefficient matrix based on SDS-PAGE was used to construct a dendrogram, in which thirty-two lentil genotypes of species *L. culinaris* were categorized based on their SDS-PAGE banding pattern. On the rescaled distance of 20 the dendrogram is divided into 10 different clusters. The cluster I comprises twelve lentil genotypes, namely IC521438, IC274090, IC398793, IC424864, IC98392, IC614705, IC541007, IC317520, EC16371, NC57762, IC59038, IC98364. The lentil genotypes IC396758, EC225503 IC201564 showed similarity in polypeptide patterns on SDS-PAGE, and placed in the cluster II. Cluster III includes of 8 lentil genotypes, namely EC955432, EC78386, IC53237, IC201548, IC405259, IC201684, IC565035, IC95654. Cluster IV includes only One genotype IC53238.Cluster V comprises two lentil genotypes namely LH84-8, LH82-6, cluster VI includes only one genotype namely LH89-48. Cluster VII includes two lentil genotypes namely LL699, EC28514. Cluster VIII comprises only one lentil genotype IC201693. LL1373 genotypes doesn't showed similarity with any genotype at the range of 20 hence placed in cluster IX, and cluster X again contain only one genotype namely LL931.

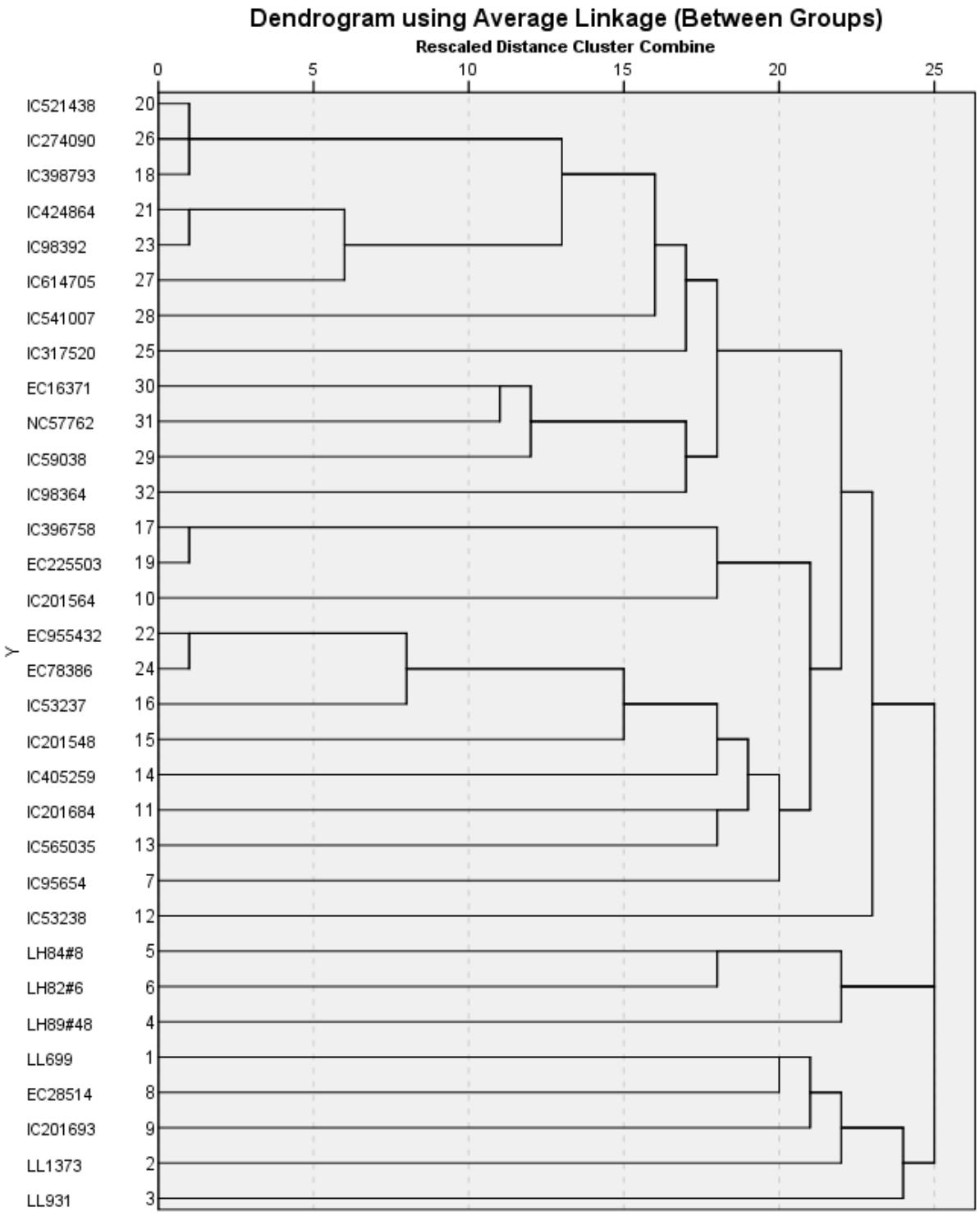

Dendrogram using Average Linkage (Between Groups)
Rescaled Distance Cluster Combine
0
5
10
15
20
25
Y
IC521438 20
IC274090 26
IC398793 18
IC424864 21
IC98392 23
IC614705 27
IC541007 28
IC317520 25
EC16371 30
NC57762 31
IC59038 29
IC98364 32
IC396758 17
EC225503 19
IC201564 10
EC955432 22
EC78386 24
IC53237 16
IC201548 15
IC405259 14
IC201684 11
IC565035 13
IC95654 7
IC53238 12
LH84#8 5
LH82#6 6
LH89#48 4
LL699 1
EC28514 8
IC201693 9
LL1373 2
LL931 3

Table 1: Electrophoretic variation of the polypeptides of the seed storage proteins of Lentil genotypes

Cluster number	Lentil genotypes	Molecular weight
I	IC521438	93, 88, 79, 71, 53, 46, 45, 40, 30, 29, 25, 22, 18, 16, 13, 11
	IC274090	93, 88, 79, 71, 53, 46, 45, 40, 30, 29, 25, 22, 18, 16, 13, 11
	IC398793	93, 88, 79, 71, 53, 46, 45, 40, 30, 29, 25, 22, 18, 16, 13, 11
	IC424864	93, 88, 79, 71, 53, 46, 45, 40, 30, 29, 25, 22, 18, 16, 13, 11
	IC98392	99, 93, 88, 79, 75, 71, 53, 46, 45, 40, 32, 29, 25, 22, 18, 16, 13, 11
	IC614705	99, 93, 88, 79, 75, 71, 53, 46, 45, 40, 32, 29, 25, 22, 18, 16, 13, 11
	IC541007	99, 88, 79, 71, 53, 46, 45, 40, 32, 23, 22, 18, 16, 11, 10
	IC317520	93, 88, 71, 70, 53, 51, 46, 45, 40, 33, 30, 29, 25, 23, 18, 16, 15, 13, 11
	EC16371	99, 93, 88, 71, 53, 48, 46, 33, 30, 25, 23, 22, 18, 16, 15, 13, 11
	NC57762	99, 88, 71, 53, 48, 46, 40, 35, 33, 30, 29, 25, 23, 22, 18, 16, 15, 13, 11
	IC59038	99, 93, 88, 79, 71, 65, 53, 48, 46, 40, 35, 30, 25, 23, 22, 18, 16, 15, 13, 11
	IC98364	99, 88, 65, 53, 51, 46, 45, 40, 35, 32, 30, 25, 24, 23, 22, 18, 15, 13, 11
II	IC396758	93, 88,75, 51, 45, 40, 33, 32, 30, 23, 22, 19, 16, 15, 12, 11
	EC225503	93, 88,75, 51, 45, 40, 33, 32, 30, 23, 22, 19, 16, 15, 12, 11
	IC201564	93, 88, 75, 60, 51, 45, 40, 32, 30, 27, 23, 22, 19, 15, 12, 10
III	EC955432	93, 88, 75, 71, 60, 53, 51, 46, 45, 40, 34, 30, 29, 25, 23, 22, 16, 14, 12, 11
	EC78386	93, 88, 75, 71, 60, 53, 51, 46, 45, 40, 34, 30, 29, 25, 23, 22, 16, 14, 12, 11
	IC53237	93, 88, 75, 71, 60, 53, 51, 46, 45, 40, 34, 30, 29, 25, 23, 22, 16, 14, 12, 11
	IC201548	99, 88, 75, 60, 53, 51, 49, 45, 39, 34, 30, 29, 25, 23, 22, 18, 16, 14, 12, 11
	IC405259	99, 88, 79, 75, 61, 53, 51, 49, 40, 34, 30, 29, 25, 23, 20, 18, 16, 12, 11, 10
	IC201684	99, 91, 75, 75, 61, 57, 53, 51, 45, 40, 34, 30, 29, 23, 22, 19, 16, 15, 12, 11
	IC565035	99, 88, 75, 61, 53, 51, 45, 42, 34, 30, 25, 23, 16, 15, 11
	IC95654	99, 91, 88, 71, 60, 53, 51, 45, 43, 39, 34, 30, 26, 23, 16, 14, 12, 11, 10
IV	IC53238	99, 91, 79, 75, 60, 57, 51, 45, 40, 33, 32, 30, 25, 23, 20, 16, 13. 11, 10
V	LH84-8	88, 84, 71, 57, 48, 40, 38, 32, 25, 24, 20, 18, 13, 12
	LH82-6	88, 84, 71, 57, 48, 40, 38, 32, 29, 25, 24, 20, 18, 13, 12
VI	LH89-48	93, 86, 84, 69, 53, 48, 45, 43, 40, 36, 32, 24, 20, 18, 13, 11
VII	LL699	93, 79, 63, 53, 51, 43, 32, 29, 20, 15, 12, 10
	EC28514	93, 79, 60, 51, 43, 32, 29, 20, 15, 13, 12, 10
VIII	IC201693	93, 84, 63, 51, 45, 43, 38, 32, 30, 19, 16, 14, 12, 11, 10
IX	LL1373	83, 79, 75, 60, 52, 48, 43, 40, 39, 38, 36, 30, 21, 17, 15, 12, 10
X	LL931	86, 77, 75, 60, 53, 43, 40, 36, 34, 32, 30, 26, 23, 22, 17, 15, 12, 11

4. Discussion

SDS-PAGE has been regarded as a potent tool for genetic diversity identification for seed storage proteins (Javaid et al, 2004). It has also been utilized to distinguish between different varieties and germplasm diversity (Nagy et al, 2009), as well as to analyse the variety of seed storage proteins in various crops (Nucca et al, 1978; Govindaraj et al, 2015). Worldwide, extensive gel electrophoresis research has also been done on seed storage proteins. Genotype characterization has been investigated in a number of crops, including wheat (Siddiqui and Naz, 2009), mustard (Geetha and Balamurugan, 2011), black gram (Ghafoor et al, 2002), *Solanum* (Mennella et al, 1999), capsicum, and *Vigna* (Rao et al, 1992), based on seed storage proteins. Madina et al, 2013 shows similar results from seed storage protein profiling of six lentil species, divided into BARI masur-1, BARI masur-2, BARI masur-3 BARI masur-4, BARI masur-5, BARI masur-6 in distinct groups. The stability of protein profiles has made the electrophoretic research of seed storage proteins an extremely useful tool, which has also been used to study the origin and development of crops (Ladizinsky and Hymowitz, 1979). Protein profiling of seed storage proteins can be used to classify germplasm, identify varieties, ascertain evolutionary relationships between species, and perform biosystematic analysis. Further, Chenbang et al. (2008) studied the SDS-PAGE profiling of total seed storage proteins for differentiating grain legumes (pigeon pea, mung bean, cow pea, black gram, common bean, lentil, pea and soybean) at species level which were grouped into three distinct groups. According to Quenum

and Yan, (2017), cluster analysis based on SDS-PAGE was consistent when cultivars under study were from the same geographic location. Fazal et al. (2012) who also observed similar findings, provide more evidence for this conclusion. Techniques for SDS-PAGE were successfully utilized to assess the genetic diversity in a variety of researches30. Tripathy et al. (2016) studied genetic variation and genetic diversity in 20 urdbean (*Vigna mungo*) genotypes using SDS-PAGE of seed storage proteins. Choudhary et al. (2015) conducted genetic diversity study of seven *Brassica napus* genotypes based on their electrophoretic pattern of seed storage proteins, and concluded that this method is effective for separating the species. Wheat endosperm protein is useful for assessing genetic variability and identifying cultivars, which aids in wheat breeding operations, according to Khan and Ali37. Moreover, Kumar and his co-workers in 2018 studied the genetic characterization in 14 lentil genotypes using SDS-PAGE, which results in distinct banding patterns and genotypic finger printing. Similarly, Manivannan, 2017 examined 21 pearl millet cultivars based on the seed storage-protein profiles on SDS-PAGE and proposed it as an effective way to separate the variants based on the electrophoretic patterns. It is possible to obtain more different genotypes between species utilizing electrophoretic patterns of the total seed storage protein, which is helpful for plant breeding programs and the evaluation of agricultural germplasm.

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