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Comparative protein profile analysis by SDS-PAGE of different grain cereals

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Abstract
The protein content and SDS-PAGE of grain storage proteins in twelve genotypes of three different cereals (wheat, barley and oats) were studied. Average grain protein (%) of wheat, barley and, oats was 10.85, 10.20 and 11.09 respectively. The twelve genotypes were clearly differentiated based on the protein banding patterns generated through SDS-PAGE. The predominant proteins of cereals resolved into three groups of different molecular weight range (high, low and mid). Interestingly, it was observed that there is good distribution of mid-range molecular weight protein (75 – 25 kDa) in cereals, whereas heavier proteins (>75 kDa) were absent in all the genotypes of wheat, barley and oats. The range of molecular weight of different proteins was observed to be 25 to 71 kDa. The dendrogram obtained using UPGMA method exhibited two major clusters. Wheat and barley were present in one cluster whereas, oat genotypes were placed in a separate cluster. The clustering pattern exhibited higher similarities among the genotypes. The similarity indexes among the different genotypes of cereals were 57 per cent, 63 per cent and 71 per cent respectively.

Keywords: SDS-PAGE, cereals, protein profile, similarity index

1. Introduction
Cereals belonging to the family Gramineae which usually have long and thin stalks. Cereal crops have been used either directly for human consumption or indirectly via livestock feed since the beginning of civilization. Grains have been the most important food source of the Indian population and different grains form staple diets of people in different part of the country. They are grown for their highly nutritious edible seeds referred to as grains (McKevith, 2004) [14]. Grain is also called a caryopsis (type of fruit), composed of the endosperm, germ and bran. They are a rich source of vitamins, minerals, carbohydrates, fats, oils and protein in whole grain form but when refined by the removal of the bran and germ, the remaining endosperm is mostly carbohydrate and lacks the majority of the other nutrients.
Cereal proteins have important nutritional and functional roles, they have been intensively studied for many years. Proteins can be used as biomarkers and if properly analysed and studied leads to identification of candidates with nutritional, industrial or medicinal value/applications. Cereal grains contain hundreds of different protein components which are traditionally classified into four so-called Osborne fractions: albumins soluble in water, globulins soluble in salt solution, prolamins soluble in aqueous alcohol and insoluble glutelins, which are only alcohol soluble. The common names of these closely related gluten proteins are gliadins (prolamins) and glutenins (glutelins) of wheat, secalins of rye, hordeins of barley and avenins of oats in the presence of reducing agents (Schalk *et al*., 2017) [18].
SDS-PAGE is quite a useful tool for distinguishing and determining genetic similarities among varieties/cultivars. It was first introduced for the separation of wheat proteins by Bietz and Wall (1972) [4]. Since then, SDS–PAGE has been widely used for separating cereal proteins from all cereals. SDS–PAGE provides a relatively low cost, high throughput method for analyzing cereal proteins. Seed proteins being stable are not affected by environmental factors hence electrophoretic analysis of seed proteins can be used for varietal identification. Diversity among cultivars can be identified and characterized by analyses of seed storage proteins and phylogenetic relationship among the accessions can be understood.
Some information is available on isoenzymes, and protein polymorphism of cereal grains (Salmanowicz and Przybylska, 1992; Dvoracek *et al*, 2003; Kumar and Matta, 2011 and Zilic *et al*, 2011) [17, 6, 8, 19]. But, a comparative study of important cereal crops is not well demonstrated. Hence, it is important and desirable to understand the genetic or protein relationships among the different cereals.

The present study was undertaken to understand the genetic diversity, relationships and identifying significant differences among the protein profiles of total proteins of four genotypes each of three different important cereals (wheat, barley and oats). SDS-PAGE technique was employed to study the total grain proteins.

2. Material and methods

The research material comprising four potentially superior genotypes/varieties from each of the three selected cereal crops viz. barley, oats, wheat was procured from Department of Crop Improvement, College of Agriculture, CSKHPKV, Palampur (Table1). The seed samples were stored in air tight containers for avoiding oxidative denaturation and further biochemical analysis. Various genotypes were analyzed in triplicate for crude protein, content by following the AOAC 2010 [1] method.

2.1 Protein profiling and phylogenetic analysis (Laemmli 1970)

Seeds were grounded using pestle and mortar; extraction of seed protein was carried out with 10 ml 0.1 M phosphate buffer (pH 7.2), 0.5 ml β- mercaptoethanol and 12 mg PVP (HiMedia, Mumbai, India). The contents were centrifuged at 18,000 rpm for 10 mins. The supernatant was collected and used for protein profiling. Protein estimation was done by Lowry's method using bovine serum albumin (BSA) as a standard (Lowry *et al*. 1951) [12]. The sample was prepared for SDS-PAGE in the sample buffer. The sample extract and buffer (50 µl each) was taken in 1:1 ratio in eppendorf tube and boiled for 3-5 mins. Total proteins were resolved on 5% stacking (pH 6.8) and 12% separating (pH 8.8) of 90 × 80 × 1mm SDS-PAGE (ATTO AE-6530m PAGE system). The 20µl samples were loaded in each well along with 10 µl protein marker. Electrophoresis was performed first at 100 V and later at 80 V in a cold room. The gel was submerged in a gel fix solution (250mg Coomassie Brilliant Blue R-250, 50% methanol, 10% glacial acetic acid and 40% deionised water) overnight. The gel was washed with destaining solution (25% methanol, 10% glacial acetic acid and 65% deionised water) The successive changes of destaining solution were given till the Coomassie dye was removed out of the gel and only the protein bands remain blue. The Coomassie stained gel was documented using Gel DocTMXR+ (BIO-RAD) Image Analyzer.

Construction of a dendrogram was done according to 'Unweighted Pair Group Method and Arithmetic Mean' method (UPGMA; Michener and Sokal, 1957) [15] using statistical software MVSP (multivariate statistical package). The gels were scored as presence (+) or absence (-) of protein polypeptide bands. Similarity index (SI) between the genotypes was calculated by the following formula:

$$SI = (2Z/X + Y) \times 100$$

Where, Z = Number of similar bands between the genotypes, and X+Y = Total number of bands in the two genotypes compared.

3. Results and Discussion

The grain protein content of four genotypes each of three different cereals is listed in Table 1 was evaluated (at least three replications each). On an average the grain protein content (%) of wheat, barley and oats was 10.85, 10.20 and 11.09 respectively (Table 2). In addition to estimation of grain protein content, SDS-PAGE profile analysis of twelve genotypes was also carried out (Fig. 1). The same amount of protein of each variety of different crops was loaded for performing the SDS-PAGE analysis. Significantly different banding patterns were observed among the different genotypes evaluated.

Table 1: Taxonomic detail of the three cereal crops

Common name	Botanical name	Tribe	Chromosome Number
Wheat	*Triticum aestivum*	Gramineae/Poaceae	42
Barley	*Hordeum vulgare*	Gramineae/Poaceae	14
Oats	*Avena sativa*	Gramineae/Poaceae	42

The range of molecular weight of different proteins was observed to be 25 to 71 kDa. The predominant proteins of cereals resolved into three groups of different molecular weight range (high, low and mid). Interestingly, it was observed that there is good distribution of mid-range molecular weight protein (75 – 25 kDa) in cereals, whereas heavier proteins (>75 kDa) were absent in all the genotypes of wheat, barley and oats. In barley, same banding pattern were observed in all four genotypes except BHS-400, in which protein band of molecular weight 47.9 kDa was not detected. In wheat, protein band of molecular weight 57.5 kDa and 44.7 kDa were not appeared in Him Pratham whereas in case of TP-40, lesser number of bands were observed. A total of 104 protein bands were identified by coomassie staining. The genotypes showed considerable variation in protein band number ranged from 3-30. Among the twelve genotypes DH-40 and Dhelu-W showed maximum number (11) of protein bands while the minimum numbers (6) of bands were present in genotypes PLP-1 and VLB-118.

Phylogenetic relationships among the six different arid legumes were estimated using UPGMA-Dice similarity index. The dendrogram obtained using UPGMA method exhibited two major clusters (Figure 2). Wheat and barley formed one cluster whereas, oat genotypes were placed in a separate cluster. The clustering pattern exhibited higher similarities among the genotypes rather than in crops. The oat and barley were found to be with least similarity. Among the wheat genotypes DH-40 and Dhelu-W formed separate sub-clusters (Figure 2) whereas Saptdhara and Him-Pratham were in single sub-cluster, which indicates that Saptdhara and Him-Pratham were phylogenetically much closer than DH-40 and Dhelu-W. In case of barley genotypes, Dolma was present as a separate sub-cluster whereas BHS-400, VLB-118 and HBL-113 were in a single sub-cluster with a similarity index of between BHS-400 and VLB-118, between BHS-400 and HBL-113 and between VLB-118 and HBL-113. The dendrogram of oat genotypes exhibited two major sub-clusters (Figure 2) with TP-40 and PLP-15 in one sub-cluster and PLP-1 and PLP-19 in the other. Based on the dendrogram it can be deduced that wheat is close to barley as compared to oat. Further, similarity index among the twelve genotypes was estimated (Table 3). The similarity index among the different

varieties of barley, oats and wheat was 57 %, 63 % and 71 % respectively (Table 3). The estimated average similarity index among the different cereal crops was highest between wheat and barley (43%) whereas it was lowest between barley and oats (20%).

While cereal grains are important sources of energy, cereals are also a primary provider of protein. The grain protein content within four genotypes of each crop varied significantly from 9.25 to 11.15 per cent in barley, 10.88 to 11.43 per cent in oat and 9.25 to 11.70 per cent in wheat as in accordance with Makeri *et al*. (2013) [13], Biel *et al*. (2014) [3] and David *et al*. (2015) [5] respectively. The grain protein values of different cereals obtained in this study (Table 2) are in agreement with earlier reports.

A total seed protein profile of different cereals is presented in the Fig. 1. The profiles of three crops are evidently different and each of the crop has a different protein banding pattern. Further, in addition to differences among the three crops, some intra-varietal differences in protein profiles were also observed (indicated in Fig. 1). Therefore, SDS-PAGE of total seed protein profiles is a useful technique for studying diversity of cereal grain. The usefulness of SDS-PAGE has been confirmed by several researchers especially with regards to taxonomy, evolution and genetic relationships among different species (Ladizinisky and Hymowitz, 1979; Ladizininky and Van Oss, 1984) [10, 9]. Further, the morphological differences observed are well supported by the dendrogram (Fig. 2) prepared by quantifying the protein bands using UPGMA method. The twelve genotypes were clustered into two major clusters: (i) Cluster 1 - with oat genotypes (ii) Cluster 2 – wheat and barley genotypes (Fig. 2). The similarity index matrix for the four genotypes of three crops clearly indicated the closeness among the varieties of individual crops (Table 3). Further, based on the similarity index of varieties, average similarity index among the crops has been

Table 2: Protein content of different cereal crops

Cereals	
Genotypes/Varieties	**Crude Protein Content (%)**
Barley	
BHS-400	10.34
Dolma	11.15
HBL-113	10.07
VLB-118	9.25
Oats	
PLP-1	10.88
PLP-15	10.88
PLP-19	11.15
TP-40	11.43
Wheat	
DH-40	9.25
Him-Pratham	11.15
Saptdhara	11.70
Dhelu-W	11.29
Cereal Crops	
Barley	10.20
Oats	11.09
Wheat	10.85
SE(±m)	*0.17*
CD (5%)	*0.66*

calculated (Table 4). This to an extent supports the results obtained from SDS-PAGE and dendrogram. As observed in the dendrogram, the highest similarity index (0.43) was observed between wheat and barley. Based on our results, the extent of relatedness was lowest between barley and oats (Table 4). Similar study was also conducted by Ahmed (2008) [2] in forty one genotypes of wheat (*Triticum aestivum* L.) representing local landraces, candidate lines and commercial cultivars. The results revealed a low level of genetic diversity

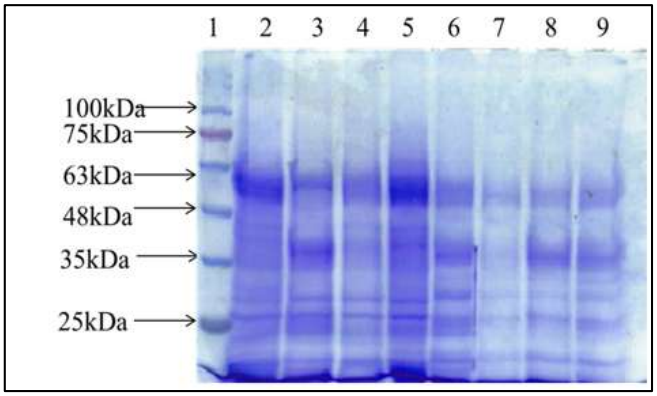

Fig 1a: SDS PAGE of Wheat and Barley Protein. L1: Standard Marker Wheat: L2: DH-40 L3: Saptdhara L4: Himpratham L5: Dhelu-W Barley: L6- Dolma L7: BHS-400 L8: VLB-118 L9: HBL-113

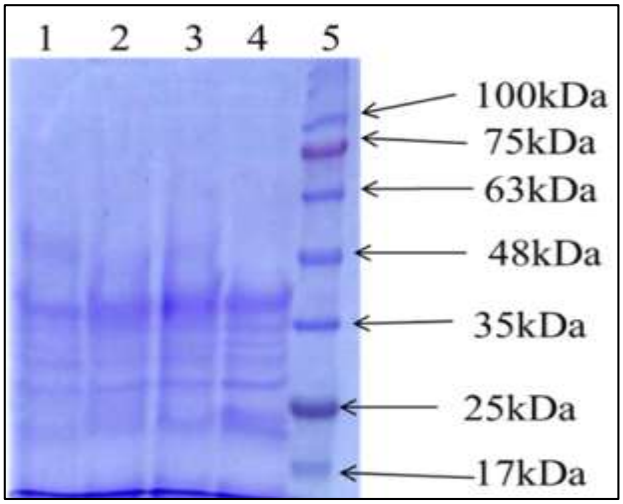

Fig 1b: SDS PAGE of Oat Protein. L1: PLP-19 L2: PLP-1 L3: PLP-15 L4: TP-40

Table 3: Similarity Index table for Cereal Genotypes

	DH-40	Saptdhara	Him Pratham	Dhelu-W	Dolma	BHS-400	VLB-118	HBL-113	TP-40	PLP-15	PLP-1	PLP-19
DH-40	1	0.57	0.6	0.55	0.48	0.47	0.53	0.4	0.2	0.11	0.24	0.33
Saptdhara		1	0.74	0.57	0.5	0.5	0.44	0.53	0.11	0	0	0.12
Him Pratham			1	0.7	0.32	0.53	0.47	0.44	0.11	0	0	0.13
Dhelu-W				1	0.38	0.35	0.32	0.3	0.1	0.21	0.12	0.22
Dolma					1	0.63	0.78	0.74	0	0.11	0	0
BHS-400						1	0.86	0.8	0.13	0	0	0
VLB-118							1	0.82	0.12	0	0	0
HBL-113								1	0.11	0	0	0
TP-40									1	0.71	0.53	0.5
PLP-15										1	0.57	0.53
PLP-1											1	0.92
PLP-19												1

which may be attributed to narrow genetic base of a wheat crop. Gregova *et al*. (2015) [7] also concluded that the genotypes of oat cultivars could effectively be differentiated on the basis of polymorphism detected between protein patterns.

Table 4: Average Similarity Indexes among different cereals estimated based on UPGMA

	Wheat	Barley	Oats
Wheat	1	0.43	0.25
Barley	-	1	0.02
Oats	-	-	1

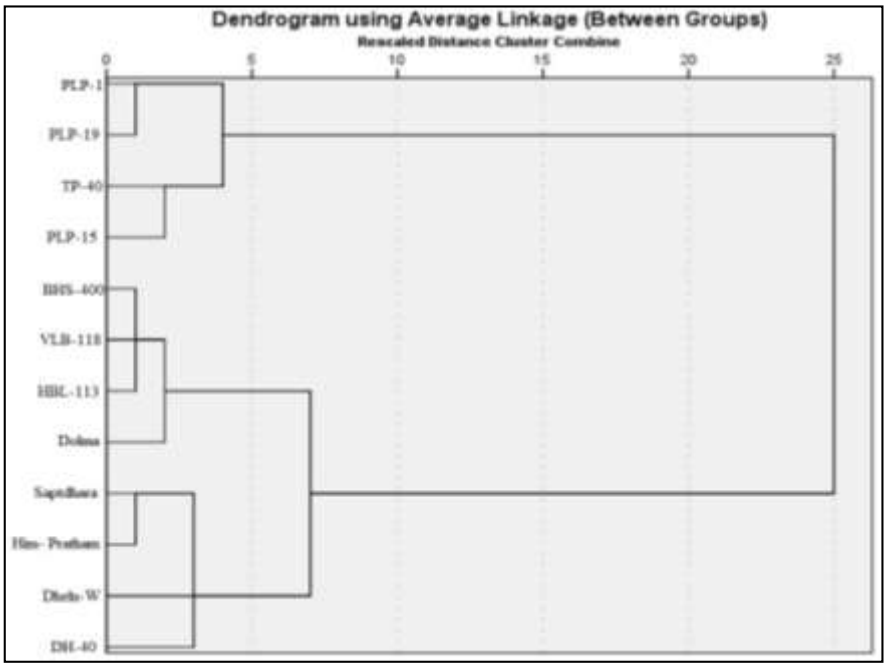

Fig 2: Dendrogram of 12 Genotypes of Selected Cereals Based on SDS-PAGE

4. Conclusion

Intra-varietal and inter-specific variability in grain protein content was observed. This variability can be useful in varietal identification and if exploited properly could be useful in breeding for high grain protein cultivars/varieties. Based on study it can be concluded that SDS-PAGE technique if done efficiently can be a useful tool in understanding the genetic diversity and relationships among different crop species/varieties.

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