Seed storage protein variation in Indian barley using 2-D diagonal gel electrophoresis approach

Jitendra Kumar Sharma
Maharshi Dayanand University Rohtak
Monika Sihmar
Maharshi Dayanand University Rohtak
Anita Rani Santal
Maharshi Dayanand University Rohtak
Nater Pal Singh (npsingh.cbt@mdurohtak.ac.in)
Maharshi Dayanand University https://orcid.org/0000-0003-3778-8711

Research Article

Keywords: 2D-diagonal gel electrophoresis, barley, seed storage protein, disulfide linkage

Posted Date: September 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-816544/v1

Abstract

Barley is the fourth most important cereal, which is used as feed and fodder. It is also used as raw material in the brewing industry and is rich in soluble dietary fiber. Thus, it has economic as well as health benefits. The present study studied seed storage protein characterization of malt, feed, two row, six row, salinity tolerant and susceptible Indian barley genotypes. The polymorphic bands in feed and malt barley were 59% and 54%; in two and six-row barley 35% and 59%; and in salt-tolerant and susceptible barley 38% and 59%. The salt-tolerant lines DL 88 and NB 1were exhibited five interlinked polypeptides, while NDB 1173, NB 3, and RD 2552 exhibited four, two, and one interlinked disulfide linkage, respectively. Whereas, in the case of salt susceptible lines Alfa 93, NB 2, K 508, BH 393 and K 551 a total of six each in Alfa 93 and K 551 barley lines, while in K 508 BH 393 barley lines, two disulfide-linked polypeptides were found. The current study portrayed a simple and economical procedure to characterize the seed protein by gel electrophoresis. Characterization of seed storage proteins by banding pattern analysis using one dimensional SDS-PAGE is the simple molecular technique to evaluate the barley lines with particular traits such as salinity tolerance ability, malt or feed quality, and others. The unique protein bands can also be used as a marker to identify the lines.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any authors.

Introduction

Barley*, Hordeum vulgare* L. is a monocotyledonous annual herb that belongs to the tribe Triticeae and is evolutionary closely related to wheat and rye (Von Bothmer and Komatsuda 2011). Barley is the fourth most important cereal in total world production after wheat, rice, and corn (Bilgi and Çelik 2004). It is cultivated around the globe as an important crop, is mainly used for fodder and as raw material for malt production, and can be typically grown in a climatically marginal area with low input. Due to the higher value of soluble dietary fiber in barley and its proven health benefits become the main part of our diet (Ceccarelli 1996; Baik and Ullrich 2008). It has been classified as spring or winter barley, two-row or six-row barley, hulled or hull-less barley due to the presence or absence of hull that tightly adhered to the grain, and feed or malting by end-use type (Ceccarelli 1996).

The industrial process of malt extraction is a standard process, but malt obtained from the batches is of different quality, and the differences may be due to variations in various factors such as cultivars, growth conditions, and storage of grains (Østergaard et al. 2004). The seed protein content of barley varies from 8 to 15% and is an important factor in deciding the end-use (Cai et al. 2013; Kumar et al. 2017; Mahalingam 2017). The breakdown of proteins into amino acids is essential as the yeasts use it for their

growth during fermentation, but it is also reported that high protein content has a detrimental effect on malt quality and quantity (Qi et al. 2005). The malting properties of barley grain are protein-dependent, and the grains with higher protein content produce lesser alcohol. Grains with high protein content can take water more slowly than grains with low protein, resulting in increased steeping time and un-uniform germination, which affects alcohol production. The wort filtration, beer haze, and beer shelf life also depend on the quantity present in the barley grains (Luo et al. 2019).

In the present study, the total seed storage protein of 34 Indian barley genotypes was analyzed by the one dimensional polyacrylamide gel electrophoresis (SDS-PAGE) for their protein characterization polypeptide patterns and genetic diversity of 34 Indian barley lines using total seed storage protein profiling. In addition, the 2D-Diagonal polyacrylamide gel electrophoresis of salt-tolerant and salt susceptible genotypes was carried out to analyze the polypeptide subunit composition among these genotypes.

Materials And Methods

Materials

Seeds of 34 barley genotypes were kindly supplied by the Indian Institute of Wheat and Barley Research (IIWBR), Karnal, Haryana, India. The barley genotypes and their pedigree are listed in Table 1.

Extraction of total seed protein and SDS-PAGE analysis

The total seed protein was extracted from the mature seeds of barley as per methods followed by Eggert and Pawelzik (2011). Barley seeds were dehulled and then ground to a fine powder using pestle and mortar. 100 mg of seed meal was taken and defatted with 10 ml of n-Hexane. The powder was vacuum dried, and proteins were extracted in protein extraction buffer (40mM Tris-Cl, pH 6.8, 15% Glycerol, 2% SDS). The concentration of proteins was estimated using a standard Bradford assay (Bradford 1976). The one-dimensional polyacrylamide gel electrophoresis of the protein sample was carried out on 10% gels according to the method (Laemmli 1970). Following gel electrophoresis, gels were stained with Coomassie brilliant blue G-250 to visualize the protein bands. The polypeptide bands produced in the electropherogram were scored, and their molecular weights were compared with the known protein standard (Himedia, MBT092).

Seed storage protein fractionation

Proteins fractions of barley seed were carried out by the method given by Shewry et al. (1978); Linko et al. (1989) with few modifications. 100 mg of seed meal used for extraction of protein fractions. Albumin (water-soluble) was extracted in autoclaved distilled water at 4℃ for 1 hour with frequent vortexing and then centrifuged, and the supernatant was collected in a fresh tube; the step was repeated twice. Globulin (salt soluble) was extracted from the residual pellet, dissolving in 1M NaCl solution at 4℃ for 1 hour with frequent vortexing followed by centrifugation at 23000g, supernatant collected in a fresh tube. Prolamin (Hordein) fractions were extracted from the residual pellet using the solution of 55% propanol with 2% 2-

mercaptoethanol at 60℃. Glutelin fractions were extracted in alkaline condition using 50mM borate buffer (pH 10.0) with 1% SDS and 0.6% 2-mercaptoethanol.

2D-Diagonal-polyacrylamide gel electrophoresis

Two-dimensional gel electrophoresis was carried out according to the method (Singh and Matta 2008). First, the proteins were separated under non-reducing conditions in the first dimension, and the gel strip was equilibrated in the equilibration buffer (0.2 M Tris–HCl, 2% SDS, 2% mercaptoethanol, pH 6.8) and loaded onto another polyacrylamide gel and run for the second dimension.

Polypeptide polymorphism analysis

The protein profile of each variety was analyzed, polymorphic bands were recorded, and percentage polymorphism of bands was calculated according to (AL-Huqail and Abdelhaliem 2015).

Polymorphism % = (No of Polymorphic bands/Total number of bands) × 100.

Statistical analysis and phylogenetic relationship

Gels were scanned with a scanner, and to record the variation in banding pattern, electropherograms of each variety were scored for presence or absence of bands in binary number as present (1) and absent (0). Next, the similarity matrix indices were constructed based on Jaccard's similarity coefficient (J) using the binary data according to the method (Singh and Matta 2008) described. Finally, a dendrogram was constructed based on the presence or absence of the band using the unweighted pair group method with arithmetic mean (UPGMA).

Results

Electrophoretic variations in polypeptide patterns in total seed storage protein

The total seed protein extracts of 34 Indian barley lines were separated on SDS-PAGE under non-reduced and reduced conditions, variation in polypeptide pattern was analyzed for their comparative studies. The polypeptides, under non-reduced conditions, were ranged between the mol wt. 14.5- 108 kDa, while the polypeptides under reducing conditions were resolved in the range of mol wt. 8-98 kDa (Fig. S1a and S1b). Some of the polypeptides of mol wt. 108, 74.5, 74, 68, 48, and 40 kDa were observed in non-reducing gels but were reduced and disappeared in the reducing conditions. The new polypeptides of mol wt. 61, 55, 44.5, 41.5, 38.5, 38, 36, 35.0, 34, 33, 32.5, 31.5, 28, 13, 10.5, 9.5, 8.5 and 8.0 kDa were resolved under reducing conditions (Fig. S1b). The variation in polypeptide patterns among malt and feed barley varieties; among two rows and six rows barley lines; and salinity tolerant (ST) and salinity susceptible (SS) lines was observed as the molecular weight of protein bands were varying in different regions. Based on the banding profile of proteins gels were divided into five regions; zone A (73-98 kDa), zone B (53-68 kDa), zone C (37.5-51kDa), zone D (25.5-35 kDa) and zone E (8-18 kDa). The polypeptide pattern analysis in these regions revealed that region A had three polypeptide patterns A1, A2, and A3, region B

had fourteen polypeptide patterns B1 to B14, region C had eighteen polypeptide patterns C1 to C18, region D had thirteen polypeptide patterns D1 to D13, and region E had two polypeptide patterns E1 and E2 (Table S1). When the polypeptides pattern of malt and feed barley were studied on the SDS-PAGE gel, the electrophoretic profiles of malt barley showed three different polypeptide patterns in region A (A, A2, and A3), eight different polypeptide patterns each in region B (B1, B2, B5, B7, B9, B12, B13, and B14) and C (C1, C2, C4, C5, C7, C8, C13, and C15), five different patterns in region D (D2, D5, D6, D8, and D10) and two polypeptide patterns in region E (E1 and E2). The polypeptide patterns of malt and feed barley in different regions have been summarized in Table S1. In region A, the pattern of mol wt. 98, 91.5, 82.5, 73 kDa (A1) occurred only in genotype K 551, the pattern of mol wt. 93, 82.5, 73 kDa (A2) occurs in five barley genotypes Alfa 93, BCU 73, DL 88, DWRB 73, and DWRUB 52. The polypeptide pattern of mol wt. 82.5, 73 kDa occurred in barley lines DWR 28, RD 2503, and RD 2668. The polypeptide pattern analysis of two rows barley exhibited two different polypeptide patterns in region A (A2 and A3), five different polypeptide patterns each in region B (B1, B2, B5, B13, and B14) and C (C1, C2, C7, C8, and C15). The polypeptide patterns of two rows and six rows barley in different regions has been summarized in Table S2. The polypeptide pattern analysis of salinity tolerant barley displayed three different polypeptide patterns, each in region A (A1, A2, and A3), region B (B3, B6, and B8), and region C (C6, C7, and C8), two different polypeptide patterns each in region D (D2 and D6) and E (E1 and E2). The polypeptide patterns of salinity tolerant and salinity susceptible barley in different regions has been summarized in Table S3.

Based on the electropherogram of total seed storage proteins of 34 different Indian barley lines, the polypeptide polymorphism percentage was calculated and analyzed among different barley lines with different characteristics (Fig. 1). The electrophoretic banding patterns of total seed protein of malt, feed, two-row, six-row, salinity tolerant, and salinity susceptible barley lines using SDS-PAGE demonstrated the differences among them (Table S4). The differences or polymorphism in the protein was based on the appearance of some bands in few varieties and the disappearance of the same bands in few varieties, whereas few bands equally appeared in all varieties of barley, i.e., monomorphic bands. SDS-PAGE analysis revealed that a total of 37 polypeptides bands of different molecular weight ranging between 8 kDa to 98 kDa together in all barley lines were present, out of which 54%, 59%, 35%, 59%, 38%, and 59% polypeptides bands were polymorphic in malt, feed, two-row, six-row, salinity tolerant and salinity susceptible barley lines respectively. However, the percentage of monomorphic bands in malt and feed barley were 43% and 41%, in two-row and six-row barley 49% and 41%, in salinity tolerant and susceptible barley 46% and 41% respectively. The polymorphic bands of 98 and 91.5 kDa of zone A appeared only in K 551, NB 1, NB 2, and Azad but were absent in two-row barley. In zone B, the polymorphic band of 65 kDa was present only in DL 88, RD 2552, and RD 2660 but absent in all two-row barley along with 54 kDa protein band, 54 kDa protein band was present in NB 2, RD 2052, RD 2503, and K 551. Another protein band of 55.5 kDa of this zone was absent in all salt-tolerant barley lines and 54 kDa protein, 55.5 kDa protein band was present only in Azad, k 560, HBL 316, DWRUB 52, DWRB 73. A polymorphic protein band of 51 kDa was absent in all salinity tolerant barley lines in zone C. In zone D, the polymorphic protein band of 28 kDa was absent in all-malt, six-row, and salinity tolerant barley lines but present in the feed, six-row, and salinity susceptible barley lines that include the PL 419, NB 2, RD 2592, and RD 2624.

The protein band of 8.5 kDa was present in all barley lines, including NB 1, NB 3, RD 2552, RD 2668, K 551, K 560, K 603, BCU 73, and DWR 28.

Electrophoretic variations in the polypeptides of hordein fractions

SDS-PAGE analysis of the hordein fractions demonstrates variations between salt-tolerant and susceptible salt lines, between two-rows and six-rows, and between malt and feed barley lines. In this study, variations were observed in hordein polypeptides from seeds of cultivars studied. Arrays of 13 to 20 polypeptide bands were observed per genotype (Fig. 2). The band size of the hordein fraction was ranged from 31 to 96.5 kDa. Other studies of hordein fractions by Lee et al. (2010) reported the fraction ranges between 31-97.4 kDa, by Binott et al. (2017) ranged between 33-96 kDa). When SDS-PAGE gel were observed polypeptide bands in salt-tolerant line DL 88 (88.0 kDa, 83.5 kDa, 47.0 kDa, and 45.0 kDa), NDB 1173 (93.0 kDa, 88 kDa, 83.5 kDa, 47 kDa, 39 kDa, and 32 kDa), NB 3 (88 kDa, 83.5 kDa, 36.5 kDa, 47 kDa, and 45 kDa), NB 1 (88 kDa, 83.5 kDa, 36.5 kDa, 47 kDa, 45 kDa, and 31 kDa). Polymorphism in polypeptides patterns was observed in the region of C-hordein and D- hordeins. The salt-tolerant lines protein amount in B-hordein and γ- hordein was less than salt susceptible lines. Variations in B-hordein among barley lines were observed. Some lines share a similar pattern in B-hordein among groups but also a variation to other groups, B- hordeins of DWR 28, DWRUB 52, K 551, K 560, PL 419, PL 426, PL 751, and RD 2508 exhibit variations among B-hordeins of HBL 113, NB 1, and RD 2715 exhibit similar, BCU 73, HBL 316, RD 2552, and RD 2592 exhibits similar B- hordein pattern, K 508 and K 603 showed a similar pattern.

Phylogenetic relationship among 34 barley varieties

The dendrogram was constructed through cluster analysis which suggests a remarkable diversity among 34 Indian barley varieties. The varieties studied were grouped into seven non-overlapping clusters (Fig. S2). In cluster IV, a maximum number of varieties constituted 12 NDB 1173, PL 751, PL 426, DL 88, DWRB 73, RD 2035, HBL 316, RD 2668 DWRUB 52, RD 2508, JB 58, and K 560. Two varieties NDB 1173 and DL 88, were salinity tolerant. Four varieties DL 88, DWRB 73, DWRUB 52, and RD 2668 were malt varieties, while DWRB 73, DWRUB 52, and RD 2668 were two-row barley. Cluster III and V both consist of 6 varieties each. Cluster III comprises HBL 113, Alfa 93, K 603, VLB 56, PL 419, and DWR 28 barley varieties, and among these varieties, Alfa 93 and DWR 28 were two-row, malt barley variety, whereas HBL 113 was two-row and feed barley variety. Cluster V comprises VLB 85, BCU 73, BH 393, K 508, RD 2715, and RD 2592. BCU 73 was two-row and malt variety among these varieties, whereas RD 2715 was dual purpose barley. Cluster VI and VII were constituted by three varieties each. In cluster VI, barley varieties RD 2624, RD 2660, and RD 2552 were present, and RD 2552 was salinity tolerant barely. In cluster VII, barley variety NB 2, K 551, and Azad were present, and K 551 was six-row malt barley. Cluster I and II possessed only two varieties each, in cluster I, NB 1 and NB 3 were present, and both varieties were salinity tolerant six-row barley. In cluster II, barley varieties RD 2052 and RD 2503 were present, and RD 2503 was six-row malt barley.

2-D Diagonal gel electrophoresis of seed proteins

For the analysis of the polypeptide subunit composition, 2D-diagonal gel electrophoresis was carried out. Since proteins are linked with disulfide bonds, significant changes in the polypeptide patterns of seed proteins under non-reducing and reducing conditions on the 1D gel were observed. The polypeptides separated under the non-reduced condition in the first dimension followed by separation in the second dimension in reducing condition demonstrated that the polypeptides having the disulfide linkage were reduced and moved as spot off of the diagonal (Fig. 3). Cleavage of inter or intramolecular disulfide linkages of proteins by 2-mercaptoethanol resulted in altered electrophoretic mobility of polypeptides. The intra-molecular cleavage of disulfide linkage resulted in a conformational change in the single polypeptide, and their mobility may increase or decrease on SDS-PAGE. Different subunits of polypeptides are linked with disulfide bonds and when treated with 2-mercaptoethanol, these disulfide linkage breaks and protein subunits resolved as different spots in 2-D gels. The polypeptides which do not have di-sulfide linkages were resolved on the diagonal, and the proteins outside the diagonal contained thiols in close proximity to form intramolecular disulfide bonds.

Inter-molecular disulfide linked polypeptides in salt-tolerant and susceptible barley lines showed polymorphism when they were cleaved by 2-mercaptoethanol (Table 2). In salt-tolerant line NB 1, the polypeptide of mol wt. 108 kDa was cleaved into 40 kDa (B_1) polypeptide subunit while it was absent in the other four salt tolerant lines. The polypeptide of 108 kDa was cleaved into 61+40, 38 kDa (F_1+ F_2, F_3) in Alfa 93, in BH 393 and K 508, the polypeptide 108 was cleaved into 63+40 kDa (G_1+ G_2, and H_1+ H_2). The polypeptide of 68 kDa of salt-tolerant line NB 1 was cleaved into 38 kDa (B_2) polypeptide subunit, while in salt susceptible lines Alfa 93 and K 551 (I_1), it was cleaved into 38+27 kDa (F_4+ F_5), and 40 kDa (I_1) respectively. The polypeptide of mol wt. 48 kDa was cleaved into 33 kDa in all salt-tolerant and susceptible lines except K 551.

The polypeptides which were resolved above the diagonal are the intramolecular disulfide-linked polypeptides (Table 3). In the genotype DL 88 polypeptides of mol wt. 37, 31, 29.5, 27.5, and 9.5 kDa represented intra-molecular disulfide linkage that resolved above the diagonal with higher mol wt. 42, 40, 32, 29, and 11 kDa, respectively. The polypeptide of mol wt. 40, 31.5, 29.5, 27.5, and 9.5 kDa were cleaved off into 44, 38.5, 32, 29, and 11 kDa polypeptides, respectively, in NB 1 barley line. In NB 3, the polypeptides of 32 and 29 kDa were resolved above the diagonal after the breakdown of intra linked disulfide linkage of polypeptides 29.5 and 27.5 kDa. The polypeptide of mol wt. 37, 31.5, 29.5, and 27.5 kDa were cleaved off into 42, 38.5, 32, and kDa polypeptides, respectively, in NDB 1173 barley line. In RD 2552, another salt-tolerant barley line, only 37 kDa polypeptide exhibited the intra linked disulfide linkage as it cleaved off into 42 kDa polypeptide. The polypeptide 27.5 kDa was common among all salt-tolerant barley lines except RD 2552. Alfa 93 and K 551 exhibited six polypeptides in salt susceptible barley lines with intra linked disulfide linkage, while K 508 and BH 393 exhibited two each, and NB 2 exhibited only one intra linked disulfide linkage (Table 3).

Discussion

Variability in seed storage proteins in *Hordeum vulgare* L. cultivated in India for use as feed or malt industries were investigated, and cultivars were analyzed. Variations among the cultivars were observed based on protein profile using one-dimensional SDS-PAGE. The distribution pattern of polypeptide bands among the varieties contributed towards the variation among the different hull-less barley (Helm et al. 2004). The differences in the banding pattern of polypeptides of barley have also been reported in many studies, in the glutelin and prolamin fraction I and II fraction (Baxter 1981), in albumin (14–58 kDa) and globulin (14–53 kDa) fraction (Linko et al. 1989). The polymorphism in the seed storage protein profile has also been reported in previous studies; Leistrumaitė and Paplauskienė (2007) assessed the hordein composition in seventeen spring barley varieties and 32 breeding lines and reported 47.1% polymorphism in the hordein profile of spring barley varieties and 34.4% polymorphism in hordein profile of breeding lines. (Sharmila et al. 2013) analyzed the protein profile of six genotypes of sorghum and reported a total of 56 scorable protein bands, out of which 54% were polymorphic and 46 % were monomorphic. Khan et al. (2013) studied 87 rice cultivars and reported a total of 16 scorable protein bands, out of which 81% were polymorphic, and 19% were monomorphic. Bibi et al. (2017) evaluated 116 rice germplasm for total seed storage proteins diversity and reported 18 scorable polypeptide bands, out of which 44.4% was polymorphic, and 55.6 % was monomorphic.

The hordein fraction synthesizes in the starchy endosperm in barley seeds and is the chief content of storage protein. The hordein fractions were earlier been divided into B- hordein (30-45 kDa), C- hordein (45-75 kDa), D- hordein (105 kDa) and γ- hordein (35-40 kDa) by Kreis and Shewry (1992), Shewry et al. (1999), Piston et al. (2005), Naeem et al. (2006). The present study is an attempt to investigate the hordein fractions for variations among the genotypes used for malting or feed, and for dual purpose, also explore the same between salt-tolerant and salt susceptible barley lines. Binott et al. (2017) studied the 22 genotypes of Kenyan barley and reported variation in D, C, and B-hordeins among the genotypes. We also observed variation in C-hordein and D- hordeins. Tanner et al. (2019) studied the hordein accumulation in developing barley grains and reported hordein concentration is associated with the quality of baked and brewed products and the regulation of disease resistance in barley. Finally, Ebrahim et al. (2015) studied the genetic diversity in 20 Euthopian barley lines using hordeins and morphological markers.

The genetic base to develop a variety of crops depends on genetic diversity. A narrow genetic base arises from a small scale genetic diversity. Crops selected to develop new, improved variety should have broadened their genetic base, i.e., with variation in protein profile (Ahmed et al. 2008; Kumar et al. 2016). Ahmed et al. (2008) has been reported genetic diversity among 41 wheat lines using SDS-PAGE. They construct a similarity matrix using UPGMA and clustered wheat genotypes into seven different clusters. Various researcher groups were reported genetic diversity among Indian barley varieties using microsatellite markers and protein profiles of seed storage proteins. Genetic diversity in 69 six-row/ two-row Indian barley was studied by Jaiswal et al. (2010) using microsatellite marker and grouped them into 10 clusters, the salinity tolerant variety NDB 1173 NB 3 in the same cluster, while in the present study, both were in two different clusters. In another study, Yadav et al. (2015) analyzed 10 Indian barley varieties, reported diversity among the variety, and grouped them into ten separate clusters. The barley

varieties K508, RD 2035 were clustered in two separate clusters. In the present study, K 508 and RD 2035 were clustered in separate clusters that agree with the study. Genetic diversity among barley was also studied using various traits (Verma and Sarkar 2010) studied 72 Indian barley varieties using seven malt and seven-grain traits. In his study, they reported Alfa 93, HBL 113, NB 1, and NB 3 were clustered in the same cluster, RD 2668, RD 2503, DL 88, and HBL 316 were in another cluster, RD 2508, RD 2624, RD 2035, RD 2052, RD 2552, RD 2592, RD 2660, K 551 and PL 426 were clustered in the same cluster. Genetic diversity in 64 barley varieties was analyzed using yield and yield contributing traits by Kumar et al. (2016), who cluster them in eight groups. Salinity tolerant variety NB 1 and RD 2552 was placed in one cluster, and the other four salinity tolerant varieties DL 88, NDB 1173, and NB 3, were placed in separate clusters, while in the current study, NB 1and NB 3 were placed in one cluster, NDB 1173 and DL 88 were placed in one cluster, and RD 2552 was placed in one cluster. The differences in the groups may be due to differences in traits of barley studied.

Protein is a complex biomolecule constituted by peptide subunits, and these subunits are held together by intermolecular disulfide bonds. In the earlier study of wheat endosperm proteins, a glutenin protein complex composed of subunits (two large A and D subunits 52 and 58 kDa respectively and two small α and subunits of 23 and 22 kDa respectively) and held together by intermolecular disulfide bonds (Singh and Shepherd 1985). Gupta et al. (1991) have been illustrated that the occurrence of HMW albumin as both polymeric (disulfide-linked aggregates) and monomeric forms in their native state by applying the diagonal electrophoresis (unreduced x reduced). The number of disulfide-linked polypeptides in salt-tolerant lines DL 88, NB 1, NB 3, NDB 1173, and RD 2552 were 5, 5, 2, 4, and 1, respectively, and the number of disulfide-linked polypeptides in salt susceptible lines Alfa 93, NB 2, K 508, BH 393 and K 551 were 6, 1, 2, 2, and 6 respectively. The data of this study is in agreement with the study of Komatsu et al. (1993), which studied the embryo and endosperm protein of rice which possessed disulfide linkage, and reported five proteins in endosperm one protein in embryo five proteins that possessed the disulfide linkage. Sadimantara et al. (1996) were characterized the endosperm protein of immature rice (*Oryza sativa* L.) seed by two-dimensional gel electrophoresis (diagonal gel). They reported a 94 kDa polypeptide identified as pyruvate orthophosphate dikinase to consist of a single polypeptide chain without intermolecular disulfide bonds. In the present study, no peptide of this molecular weight was seen below the diagonal or above the diagonal, suggesting that barley too had pyruvate orthophosphate dikinase with a single polypeptide chain.

Conclusion

The current study portrayed a simple and economical procedure to characterize the seed protein by gel electrophoresis. Characterization of seed storage proteins by banding pattern analysis using one dimensional SDS-PAGE is a simple molecular technique to evaluate the barley lines with particular traits such as salinity tolerance ability, malt or feed quality, and others. The unique protein bands can also be used as markers to identify the lines and use as a genetic base to improve crops.

Acknowledgement

The financial assistance was provided by Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India, New Delhi under grant no. SB/YS/LS-334/2013 and SB/EMEQ-085/2014 and University Grants Commission, New Delhi under grant no. 41-515/2012 (SR) is highly acknowledged.

References

Ahmed K, Ahmed A, Abbas Z, et al (2008) Genetic diversity in wheat (*Triticum aestivum* L.) as revealed by SDS-PAGE analysis. Int J Appl Agric Res 3:1–8

AL-Huqail AA, Abdelhaliem E (2015) Evaluation of Genetic Variations in Maize Seedlings Exposed to Electric Field Based on Protein and DNA Markers. BioMed Res Int 2015:1–15. https://doi.org/10.1155/2015/874906

Baik B-K, Ullrich SE (2008) Barley for food: Characteristics, improvement, and renewed interest. J Cereal Sci 48:233–242

Baxter ED (1981) Hordein in barley and malt—A review. J Inst Brew 87:173–176

Bibi A, Rabbani MA, Abbasi FM, et al (2017) Assessment of genetic diversity in indigenous rice accessions of northern Pakistan using biochemical markers. Pak J Bot 49:155–161

Bilgi B, Çelik S (2004) Solubility and emulsifying properties of barley protein concentrate. Eur Food Res Technol 218:437–441

Binott JJ, Owuoche JO, Bartels D (2017) Physiological and molecular characterization of Kenyan barley (*Hordeum vulgare* L.) seedlings for salinity and drought tolerance. Euphytica 213:139. https://doi.org/10.1007/s10681-017-1924-2

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cai S, Yu G, Chen X, et al (2013) Grain protein content variation and its association analysis in barley. BMC Plant Biol 13:35. https://doi.org/10.1186/1471-2229-13-35

Ceccarelli S (1996) Adaptation to low/high input cultivation. Euphytica 92:203–214

Ebrahim S, Shiferaw E, Hailu F (2015) Evaluation of genetic diversity in barley (*Hordeum vulgare* L.) from Wollo high land areas using agromorphological traits and hordein. Afr J Biotechnol 14:1886–1896

Gupta RB, Shepherd KW, MacRitchie F (1991) Genetic control and biochemical properties of some high molecular weight albumins in bread wheat. J Cereal Sci 13:221–235. https://doi.org/10.1016/S0733-

5210(09)80002-7

Helm CV, de Francisco A, Gaziola SA, et al (2004) Hull-less Barley Varieties: Storage Proteins and Amino Acid Distribution in Relation to Nutritional Quality. Food Biotechnol 18:327–341. https://doi.org/10.1081/FBT-200040531

Jaiswal SK, Pandey SP, Sharma S, et al (2010) Diversity in Indian barley (*Hordeum vulgare*) cultivars and identification of genotype-specific fingerprints using microsatellite markers. J Genet 92:46–54

Khan SA, Shinwari ZK, Rabbani MA (2013) Study of total seed protein pattern of rice (Oryza sativa L.) breeding lines of Pakistan through SDS-PAGE. Pak J Bot 45:871–876

Komatsu S, Kajiwara H, Hirano H (1993) A rice protein library: a data-file of rice proteins separated by two-dimensional electrophoresis. Theor Appl Genet 86:935–942. https://doi.org/10.1007/BF00211044

Kreis M, Shewry PR (1992) The control of protein synthesis in developing barley seeds. Barley Genet Biochem Mol Biol Biotechnol 319–333

Kumar A, Bharti B, Kumar J, et al (2016) Genetic variability and divergence analysis for yield and yield contributing traits in released varieties of barley (*Hordeum vulgare* L.) under partially reclaimed saline sodic soil. J Appl Nat Sci 8:2273–2277. https://doi.org/10.31018/jans.v8i4.1124

Kumar V, Kumar L, Kharub AS (2017) Trends of Seed Production, Varietal Scenario and Future Prospects in Barley. J Wheat Res 9:. https://doi.org/10.25174/2249-4065/2017/70102

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. nature 227:680–685

Lee YJ, Lee TG, Jeon WB, et al (2010) Employment of hordein subunit polymorphisms in establishing selection criteria for high quality malting barley (*Hordeum vulgare* L.). J Crop Sci Biotechnol 13:91–97. https://doi.org/10.1007/s12892-010-0057-0

Leistrumaitė A, Paplauskienė V (2007) Genetic resources of spring barley: analysis of Hordein polymorphism. Biologija

Linko R, Lapvetelainen A, Laakso P, Kallio H (1989) Protein composition of a high-protein barley flour and barley grain. Cereal Chem 66:478–482

Luo H, Harasymow S, Paynter B, et al (2019) Genetic and environmental impact on protein profiles in barley and malt. J Inst Brew 125:28–38

Mahalingam R (2017) Shotgun proteomics of the barley seed proteome. BMC Genomics 18:44. https://doi.org/10.1186/s12864-016-3408-5

Naeem R, Ahmed I, Asghar R, Mirza B (2006) Characterization of *Hordeum vulgare* accessions of Pakistan by Hordein SDS-PAGE. Cereal Res Commun 34:1247–1254

Østergaard O, Finnie C, Laugesen S, et al (2004) Proteome analysis of barley seeds: Identification of major proteins from two-dimensional gels (pI 4–7). PROTEOMICS 4:2437–2447. https://doi.org/10.1002/pmic.200300753

Piston F, Martín A, Dorado G, Barro F (2005) Cloning and molecular characterization of B-hordeins from *Hordeum chilense* (Roem. et Schult.). Theor Appl Genet 111:551–560

Qi J, Chen J, Wang J, et al (2005) Protein and hordein fraction content in barley seeds as affected by sowing date and their relations to malting quality. J Zhejiang Univ Sci B 6:1069

Sadimantara GR, Abe T, Suzuki J, et al (1996) Characterization and partial amino acid sequence of a high molecular weight protein from rice seed endosperm: Homology to pyruvate orthophosphate dikinase. J Plant Physiol 149:285–289

Sharmila P, Sarada MN, Ratan-Kumar PK (2013) Genetic variability among genotypes of sorghum based on protein profile of seed storage proteins. Int J Adv Biotechnol Res 4:280–85

Shewry PR, Tatham AS, Halford NG (1999) The prolamins of the Triticeae. In: Seed proteins. Springer, pp 35–78

Singh NK, Shepherd KW (1985) The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. Theor Appl Genet 71:79–92. https://doi.org/10.1007/BF00278258

Singh NP, Matta NK (2008) Variation studies on seed storage proteins and phylogenetics of the genus Cucumis. Plant Syst Evol 275:209–218. https://doi.org/10.1007/s00606-008-0063-6

Tanner GJ, Colgrave ML, Blundell MJ, et al (2019) Hordein accumulation in developing barley grains. Front Plant Sci 10:649

Verma RPS, Sarkar B (2010) Diversity for malting quality in barley (*Hordeum vulgare*) varieties released in India. Indian J Agric Sci 80:493

Von Bothmer R, Komatsuda T (2011) Barley origin and related species. Barley Prod Improv Uses 14–62

Yadav SK, Singh AK, Malik R (2015) Genetic diversity analysis based on morphological traits and microsatellite markers in barley (*Hordeum vulgare*). Indian J Agric Sci 85:37–44

Tables

Table 1. List of barley germplasm and their pedigree and characteristics

Sr. No.	Barley cv.	Malt/ Feed	Pedigree	Salient Features
1	ALFA 93	Malt [d, f]	AURORA/QUEEN // BEKA [a, f]	Hulled, 2 row [d, f]
2	AZAD	Feed [c]	K 12 / K 19 [a, c]	Hulled, 6 row [c]
3	BCU 73	Malt [c, d, f]	WUM143 (YAGAN) [a, c]	Hulled, 2 row [c, d]
4	BH393	Feed [c, e]	California Mariout / RATNA [a, c, f]	Hulled, 6 row [c, f]
5	DL 88	Malt [c, d]	BG 1 / Mex 5-13 [a, c, f]	Hulled, 6 row [c, f]
6	DWR 28	Malt [c, d, f]	BCU 73 / PL 172 [a, c]	Hulled, 2 row [c, d]
7	DWRB 73	Malt [d, f]	PL710/DWR17	Hulled, 2 row [d]
8	DWRUB 52	Malt [d, f, e]	DWR 17/ K 551 [a]	Hulled, 2 row [d]
9	HBL 113	Feed [f, e]	Selection from Zyphzee [a, c, f]	Hulled, 2 row [f]
10	HBL 316	--	Mutant of HBL98 [a]	Hulled, 6 row
11	JB 58	Feed [e]		Hulled, 6 row
12	K 508	Feed	K394/K141	Hulled, 6 row
13	K551	Malt [c, d]	P 464 / JYOTI [a, c]	Hulled, 6 row [c]
14	K560	Feed [c]	K 404 / DL 479 [c]	Hulled, 6 row [c]
15	K603	Feed [c]	K 257 / C 138 [a, c]	Hulled, 6 row [c]
16	NB-1	Feed [c]	Karan15 / P408 [a, c]	Hulled, 6 row [c]
17	NB-2	Feed [c]	DL470 / RD2035 [a, c]	Hulled, 6 row [c]
18	NB-3	Feed [c]	K425 / Jyoti [a, c]	Hulled, 6 row [c]
19	NDB1173	Feed [c, e]	BYT-LRA- 3 (1994-95) / NDB 217 [a, c, f]	Hulled, 6 row [c, f]
20	PL 419	Feed [c]	PL 101 / PH 182 [a, c]	Hulled, 6 row [c]
21	PL 426	Feed [c, e]	KARAN 92 / PL 101 [a, c]	Hulled, 6 row [c]
22	PL 751	Feed [e]	K226/PL226 [a]	Hulled, 6 row
23	RD 2052	Feed [c, e]	Api-CM-67/SO-727// PL101 [a, c]	Hulled, 6 row [c]
24	RD 2503	Malt [d]	RD 103 / BH 153 // RD 2046 [a, c]	Hulled, 6 row [c]
25	RD 2552	Dual [d]	PD 2035 / DL 472 [a, c]	Hulled, 6 row [c]
26	RD 2035	Dual [d, e]	RD137/PL101 [a, c]	Hulled, 6 row [c]
27	RD 2508	Feed	RD2035/P409 [a]	Hulled, 6 row
28	RD 2592	Feed [e]	RD2503/UBL9 [a]	Hulled, 6 row
29	RD 2624	Feed [c]	BL 2 / RD 2508 [a, c]	Hulled, 6 row [c]
30	RD 2660	Feed [e]	RD2052/RD2566 [a]	Hulled, 6 row
31	RD 2668	Malt [d, f]	RD2035/BCU73 [a]	Hulled, 2 row [d]
32	RD 2715	Dual [d, e]	RD387/BH602//RD2035 [g]	Hulled, 6 row
33	VLB 56	Feed [c, f]	Morocco/VLB1 [a, c]	Hulled, 6 row [c, f]
34	VLB 85	Feed [d]	HBL 348/VLB 49 [b]	Hulled, 6 row [b]

([a]Verma and Sarkar 2010; [b]Kant et al. 2012; [c]Jaiswal et al. 2010; [d]Kumar et al. 2014, [e]Kumar et al. 2017; [f]Singh et al. 2016; [g]Verma et al. 2016)

Table 2. The polypeptide and their subunit composition in the total seed protein of salt-tolerant and susceptible barley lines.

Barley genotypes		No. of polypeptide subunit	Molecular weight (kDa)	
			Polypeptide subunit	Polypeptide subunit composition
Salt Tolerant	DL 88	1	48.0	41.5 and 32.5
	NB 1	1	48.0	32.5
		2	68.0	38.5
	NB 3	1	48.0	35.0
	NDB 1173	1	48.0	34.5
	RD 2552	1	48.0	35.0
Salt Sensitive	Alfa 93	1	48.0	31.5
		2	68.5	36.5 and 28.0
		3	108.0	61.0, 39.0, 36.5 and 31.5
	NB 2	1	48.0	36.5
		2	74.5	44.5 and 36.5
	K 508	1	40.0	38.5
		2	48.0	34.0
		3	74.0	65.0, 55.0, 42.5, 38.0, and 33.0
	BH 393	1	48.0	31.5
		2	68.0	33.0
		3	108.0	54.0 and 36.0
	K 551	1	48.0	34.0
		2	74.0	41.0

Table 3. The intra-molecular disulfide-linked polypeptide and their subunit composition in the total seed protein of salt-tolerant and susceptible barley lines.

Sr. No.	Molecular weights of the polypeptides having intra-molecular disulfide linkages (kDa)									
	Salt stress tolerant barley genotypes									
	DL 88		NB 1		NB 3		NDB 1173		RD 2552	
	NR	R	NR	R	NR	R	NR	R	NR	R
1	40.5	47.0	40.5	47.0	29.5	33.0	38.0	44.5	41.5	46.5
2	35.0	42.0	31.5	38.0	29.0	32.0	34.0	38.0	9.0	11.0
3	30.0	35.0	29.5	32.0			31.0	34.0		
4	27.5	30.5	27.5	30.5			30.0	31.5		
5	9.5	11.0	9.5	11.0			9.0	11.0		
	Salt stress sensitive barley genotypes									
	Alfa 93		NB 2		K 508		BH 393		K 551	
	NR	R	NR	R	NR	R	NR	R	NR	R
1	40.5	51.5	31.0	35.0	29.5	34.0	27.0	30.0	35.0	43.0
2	30.0	37.0					25.5	29.5	34.0	41.0
3	25.5	30.0							27.5	41.0
4	25.0	32.5							13.0	16.5
5	24.5	31.5								
6	19.0	20.0								
7	13.0	16.5								

Figures

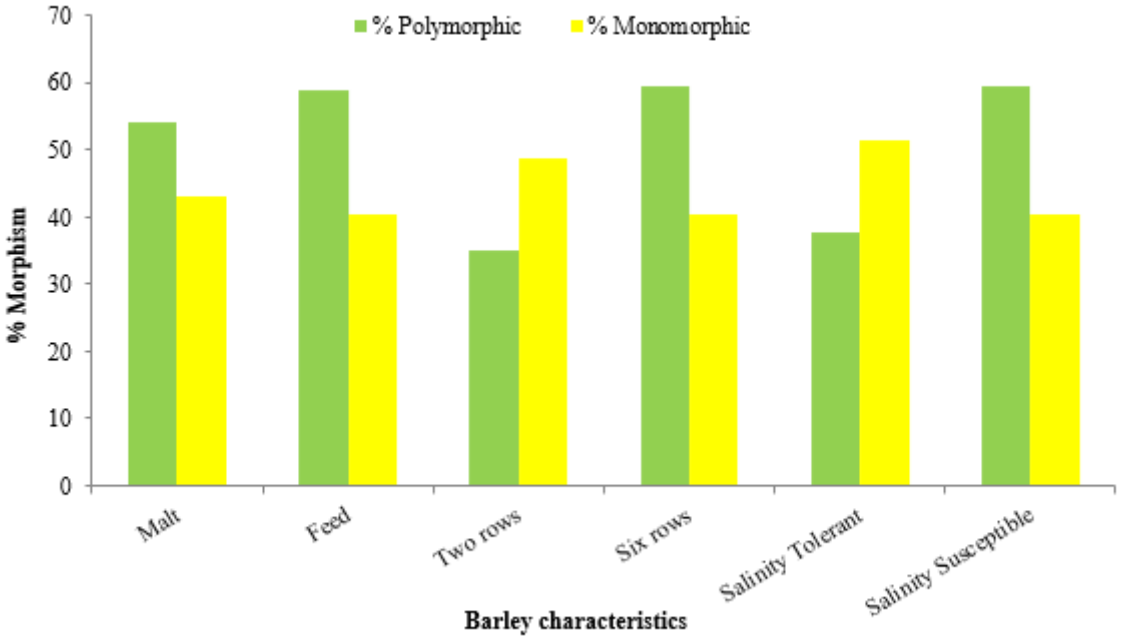

Figure 1

Polypeptide polymorphism percentage in different barley lines.

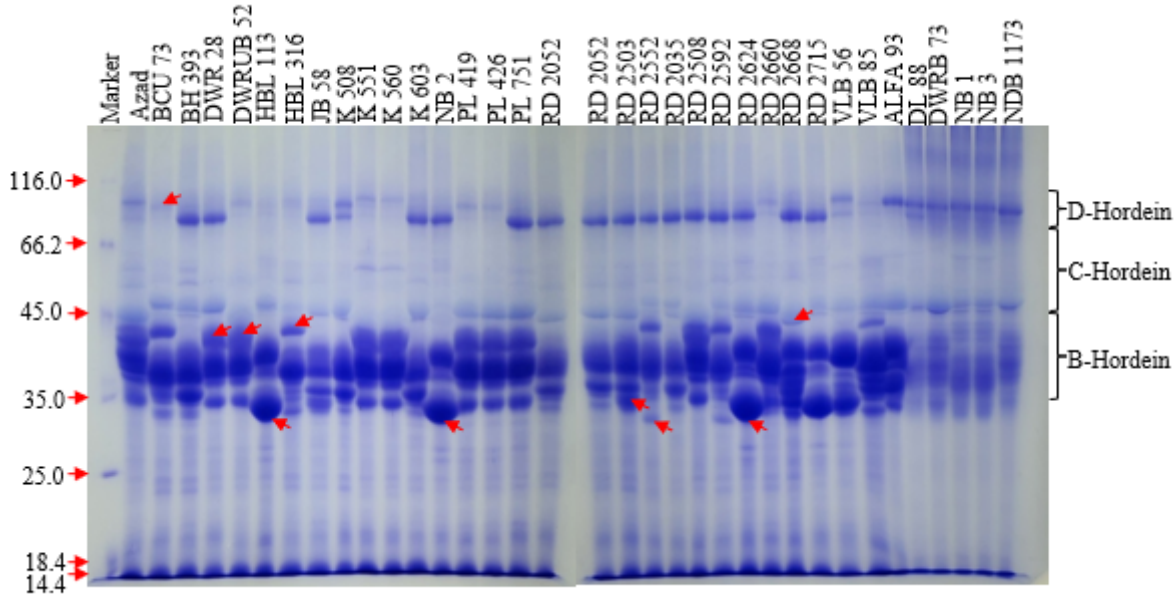

Figure 2

Analysis of barley hordein fraction polypeptides. Alcohol soluble hordein polypeptide fractions resolved using 11% SDS-PAGE. A similar pattern of B- hordeins in DWR 28, DWRUB 52, K 551, K 560, PL 419, PL 426, PL 751, and RD 2508. B-hordeins of HBL 113, NB 1, and RD 2715 exhibit similar, BCU 73, HBL 316,

RD 2552, and RD 2592 exhibits similar B- hordein patterns, and K 508 and K 603 showed similar B-hordein patterns.

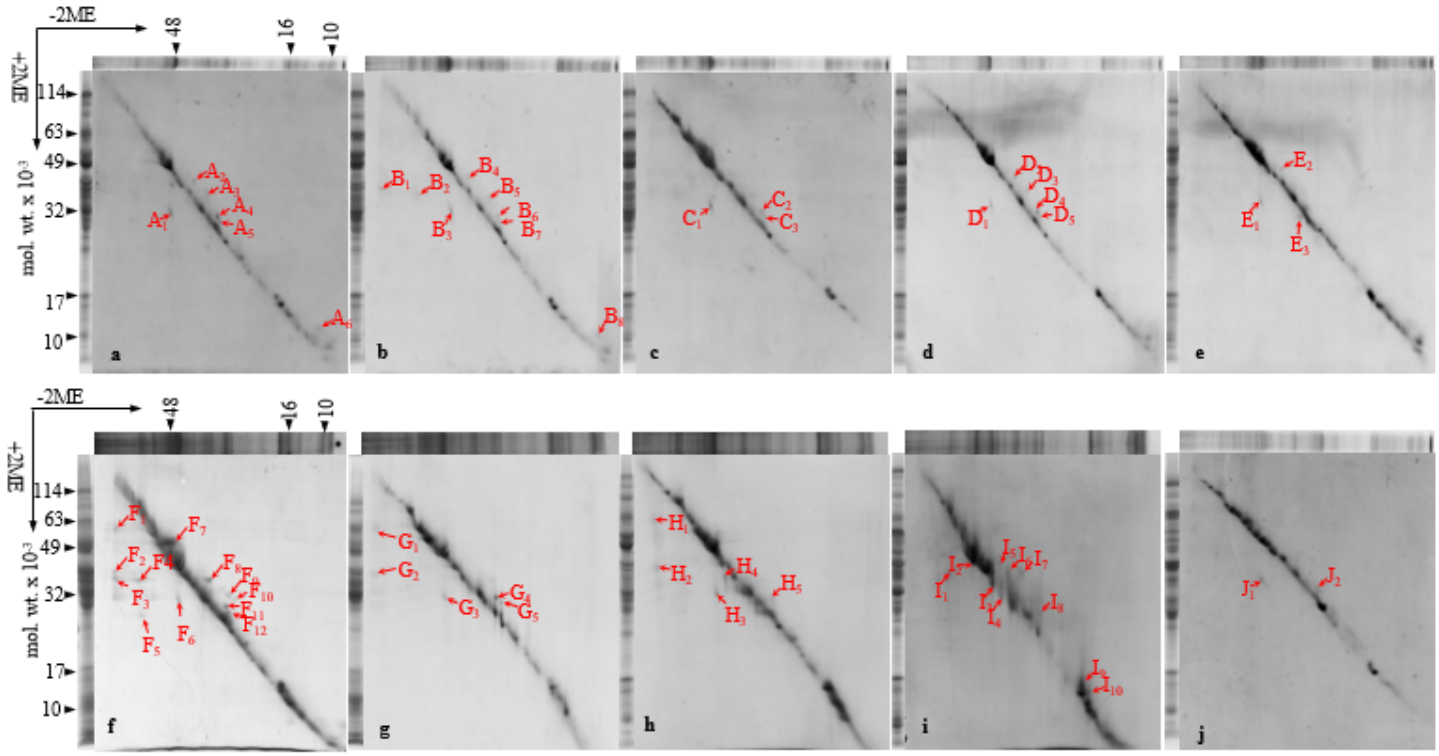

Figure 3

The 2D diagonal gel electrophoresis of total seed storage proteins of salt-tolerant and susceptible barley genotypes. The polypeptides which do not have di-sulfide linkages were resolved on the diagonal, and the polypeptides that have inter and intra-molecular disulfide linkages were resolved below and above the diagonal respectively. (a) DL 88, (b) NB 1, (c) NB 3, (d) NDB 1173, (e) RD 2552, (f) Alfa 93, (g) BH 393, (h) K 508, (i) K 551, (j) NB 2.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Attachmenttomanuscript.docx