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SHORT COMMUNICATION

Assessment of Protein Fractions as Biochemical Indicators for Differentiating *Triticum aestivum* and *Triticum durum* Wheat Varieties

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Abstract

Background: Wheat quality is determined by the composition and quantity of its protein classes, including albumins, globulins, gliadins, and glutenins. Variability in these protein profiles plays a critical role in differentiating wheat cultivars and assessing their suitability for various food products. **Aim:** This study aimed to evaluate the protein profiles of bread wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*) cultivars using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The focus was to identify polymorphisms and their relationship with dough quality. **Methods:** Seed proteins from three cultivars each of *T. aestivum* and *T. durum* were analyzed using SDS-PAGE. Protein fractions were examined to identify distinct banding patterns, and specific loci associated with dough quality, such as Glu-D1 and Glu-B1, were studied. **Results:** The analysis revealed 22 to 24 protein bands with molecular masses ranging from 2 to 250 kilodaltons, of which 13 showed polymorphism. Clear differences in banding patterns were observed between *T. aestivum* and *T. durum*, particularly in gliadin and glutenin fractions. Gliadin band 7 was present in all *T. aestivum* cultivars, while band 11 was exclusive to *T. durum*. Glutenin analysis showed that the Glu-D1 locus, marked by alleles 2+12 and 5+10, was common in *T. aestivum*, with allele 5+10 linked to superior dough quality. Conversely, the Glu-B1 locus, characterized by allele 20+8, was present in *T. durum* and associated with lower bread-making quality but better suitability for pasta production. **Conclusion:** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis is a valuable method for detecting protein polymorphism and assessing wheat quality. The study demonstrates significant variability in protein profiles between *T. aestivum* and *T. durum*, providing insights into their functional properties and food processing potential.

Major Findings: The study identified significant protein profile differences between *T. aestivum* and *T. durum*, particularly in gliadin and glutenin fractions. Key alleles linked to dough quality were found, with *T. aestivum* favoring bread-making and *T. durum* suited for pasta production.

Keywords: Bread Wheat, Durum Wheat, Glutenin Fractionation, Glu-D1 and Glu-B1 Loci Dough Quality, Varietal Identification

1. Introduction

Wheat is one of the most important cereals globally. Wheat is a valuable source of nutrients, supporting both human health and animal nutrition. Wheat contributes nearly 20 % of the global population's caloric intake. Hexaploid bread wheat accounts for roughly 95 % of worldwide wheat cultivation, while tetraploid durum wheat makes up the remaining 5 %[1]. Wheat Proteins are broken down into their individual subunits, called monomers, including gliadins, albumins, and globulins, along with polymeric glutenins. The glutenin group is composed of HMW-GS and LMW-GS[2]. Protein plays a vital role in determining the functionality of wheat flour in diverse food applications. Mature wheat grains have a protein range of 8 % to 20 %, which is highly complex and involves numerous interactions, making their analysis a challenging task. Typically, these proteins are classified based on their solubility. Albumins and globulins make up 20-25 % of wheat endosperm protein[3,4]. From a nutritional standpoint, these non-gluten proteins offer a well-balanced amino acid profile.

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A substantial proportion of these proteins function as enzymes that support metabolic processes. What sets wheat apart from other edible grains is its ability to form gluten—a protein complex in wheat flour that can be developed to create dough with a defined rheological behavior characteristic needed for producing Aerated bread[5]. The gluten's unique rheological properties are essential not only for bread making but also for a wide variety of Wheat products like noodles, pasta, and baked goods[6]. Gluten is made of gliadins and glutenins, which together make up around 75–85 % of the total protein in wheat grain, with a nearly one-to-one equivalence ratio in wheat[3,7]. These proteins are high in arginine, glutamine, asparagine and proline but low in tryptophan, lysine, and methionine[8,9].

Identification of crop varieties is a key focus for plant breeders, pathologists, seed technologists, and biochemists[10]. Polyacrylamide Gel Electrophoresis (PAGE) offers a biochemical approach to the evolutionary aspect of a plant speciation. Electrophoretic method being rapid, cheap, eliminating the need to grow plants to maturity, are used to check the purity of seeds, varietal identification and to evaluate the genetic diversity[11]. This study measures protein content and fractions in wheat in three bread and three durum wheat varieties through SDS-PAGE analysis.

2. Materials and Methods

Seeds of 6 wheat varieties namely three aestivum (GW 322, GW 366, GW 496) and three durum (PDW 291 Delhi, HI 8498 Junagadh, NIDW 295 Niphad) were studied for biochemical analysis. Three aestivum wheat varieties were selected from Regional Research Station, Arnej, Gujarat and three Durum Wheat varieties were from Kernal's directorate of wheat research.

2.1 Determination of Wheat Total Protein was using SDS-PAGE

Protein from 100 mg of wheat seed flour was made uniform in 1 mL Tris-HCl buffer (0.05 M, pH 7.2), 0.02 % SDS, and 1% β-mercaptoethanol, and kept at RT for 24 hours. Homogenate was centrifuged at 13,000 rpm revolutions per minute for 15 min at 4 degrees Celsius. The resulting clear the liquid on top was combined with gel loading dye (containing SDS) and then separated using 12 % PAGE. After running gel for 2 hours, Stained with Coomassie Brilliant Blue (0.1 %) in a methanol solution, acetic acid, and distilled water in a 40:10:50 ratio follow by destained[12].

2.2 Wheat Flour Proteins were Analyzed by SDS-PAGE

One hundred milligrams of wheat flour were homogenized in an appropriate solvent, as indicated in Table 1 and then centrifuged at 6000g for 10 minutes. The resulting filtrate was used for subsequent analysis[4].

2.3 Analysis of Electrophoresis Data

Bands were recorded as 1 or 0, respectively for presence or absence. This information was then entered into a binary data matrix as discrete variables. A dendrogram was created using UPGMA. Cluster analysis was done by NTSYSpc (version 2.2) (Numerical Taxonomy System).

3. Results

3.1 SDS-PAGE Pattern of Total Protein

Total protein was analyzed using a 12% separation gel via Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Electrophoresis revealed 22 to 24 bands with molecular weights ranging from 2 to 250 kilodaltons (kDa) (Figure 1). Of these, 13 bands were polymorphic, while 11 were monomorphic and present across all wheat varieties. Polymorphism was most evident in the molecular weight range of 50 to 150 kDa. Relative mobility bands 1 (0.20), 4 (0.26), 8 (0.32), 9 (0.36), 15 (0.51), 17 (0.58), 23 (0.74), and 24 (0.76) were exclusive to *Triticum durum* varieties, while bands 2 (0.24), 3 (0.26), 5 (0.28), 7 (0.31), and 22 (0.74) were found only in *T. aestivum* varieties. A UPGMA dendrogram based on the similarity matrix formed two primary clusters: Cluster A, comprising *T. aestivum* varieties, and Cluster B, comprising *T. durum* varieties (Figure 6). The utility of protein electrophoresis in distinguishing wheat varieties through polymorphic banding patterns[13].

3.2 Albumin Protein Fraction

The albumin protein fraction exhibited 16 to 17 bands, with relative mobility (Rm) values ranging from 0.25 to 0.83 (Figure 2). Bands 2 (0.35) and 11 (0.65) were specific to GW 366, while band 12 (0.66) was found

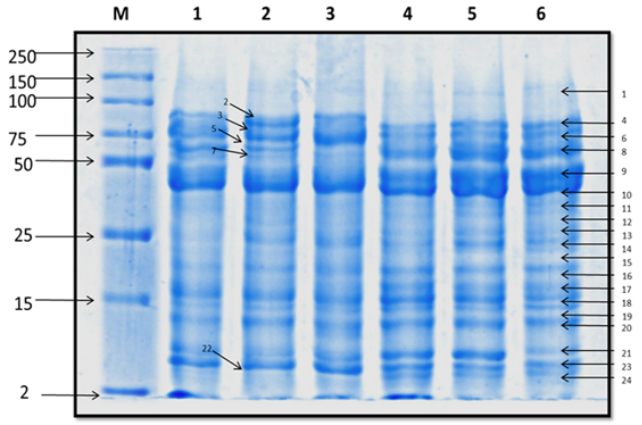


Figure 1. SDS PAGE profile of total proteins in different wheat varieties.

Table 1. Optimal temperature, duration, and solvent utilized for various protein fractions

Fractions	Temp and Time	Solvent used
Albumin	4 °C, 4hrs	Distilled water
Globulin	4 °C, 4hrs	5% NaCl solution
Gliadin	°C, 4hrs	70%ethanol

only in NIDW 295 (Niphad). Band 15 (0.76) was present in both GW 366 and PDW 291 (Delhi). Bands 3 (0.39), 6 (0.43), and 7 (0.47) were observed in all *T. aestivum* varieties but absent in *T. durum*. Conversely, band 8 (0.53) was present exclusively in all *T. durum* varieties. A dendrogram based on albumin fraction data revealed two primary clusters: Cluster A (*T. aestivum*) was further subdivided into subclusters A1 (GW 322 and GW 496) and A2 (GW 366). Cluster B (*T. durum*) was subdivided into B1 (PDW 291 and HI 8498) and B2 (NIDW 295) (Figure 7). The importance of albumin proteins in distinguishing wheat varieties has been previously noted and emphasized their potential as genetic markers for cultivar identification[14,15].

3.3 Globulin Protein Fraction

The globulin protein fraction displayed 14 bands, 2 of which were polymorphic (Figure 3). Band 8 (0.57) was exclusive to *T. aestivum* varieties, while band 9 (0.63) was absent in *T. aestivum*. Twelve bands were monomorphic and present in all wheat varieties. The dendrogram for globulin proteins formed two primary clusters: Cluster A (*T. aestivum*) and Cluster B (*T. durum*) (Figure 8). Globulin fractions exhibited fewer

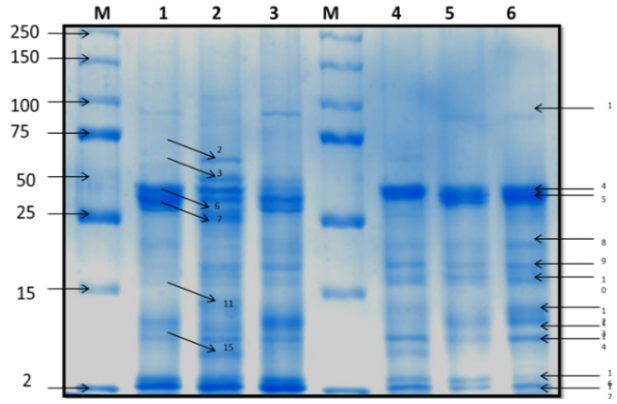


Figure 2. SDS PAGE profile of protein fraction albumin in differentwheat varieties.

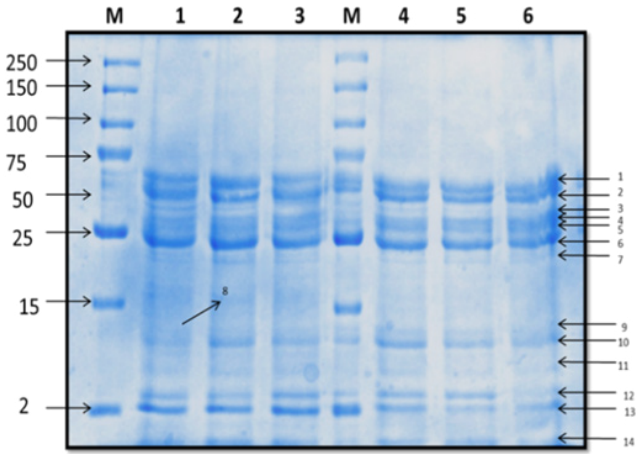


Figure 3. SDS PAGE profile of proteinfraction globulinin differentwheat varieties.

polymorphisms than albumin fractions, but the unique presence of band 8 in *T. aestivum* highlights the role of globulin proteins in distinguishing between these species[10].

3.4 Gliadin Protein Fraction

Gliadin proteins, categorized into α, β, γ, and ω fractions, had molecular weights ranging from 30 to 80 kDa (Figure 4). A total of 13 bands were detected, 9 of which were monomorphic. Band 6 (0.47) was observed in GW 366 and NIDW 295. Band 7 (0.51) was present in all *T. aestivum* varieties, while bands 9 (0.61) and 11 (0.73) were present in all *T. durum* varieties[16,17]. The gliadin dendrogram formed two distinct clusters: Cluster A (*T. aestivum*) and Cluster B (*T. durum*) (Figure 9). The polymorphism of gliadin fractions across wheat species and their relevance in distinguishing between *T. aestivum* and *T. durum*[2].

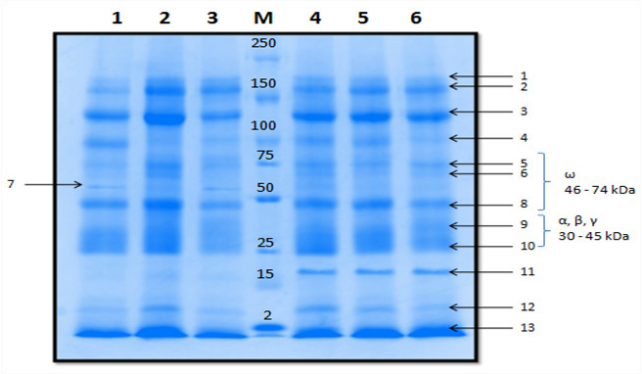
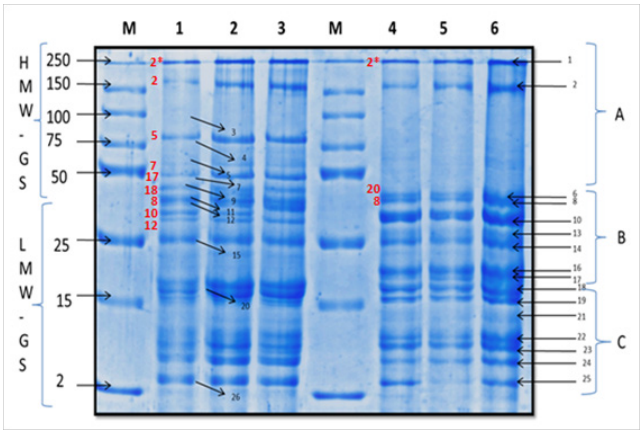


Figure 4. SDS PAGE profile of proteinfraction gliadinin different wheat varieties



M= Marker (1) GW 322, (2) GW 366, (3) GW 496, (4) PDW 291, (5) HI 8498, (6) NIDW 295

Figure5. SDS PAGE profile of HMW-GSand LMW-GS in different wheat varieties.

3.5 Glutenin Protein Fraction

Reduced and alkylated glutenin proteins were categorized into A, B, and C groups via SDS-PAGE (Figure 5). High molecular weight glutenin subunits (HMW-GS) were detected between 45 and 250 kDa, while Low Molecular Weight Glutenin Subunits (LMW-GS) ranged from 2 to 45 kDa. The HMW-GS subunit Ax 2*, which contributes to bread quality, was found in all *T. aestivum* varieties and rarely observed in *T. durum*. The Glu-D1 locus (alleles 2+12 and 5+10) was present in all *T. aestivum* varieties, with allele 5+10 linked to superior dough quality. In contrast, the Glu-B1 locus (allele 20+8) was identified in all *T. durum* varieties, contributing to inferior bread quality but superior pasta-making properties. A dendrogram based on glutenin profiles revealed two clusters: Cluster A (*T. aestivum*) and Cluster B (*T. durum*)[15,18] (Figure 10).

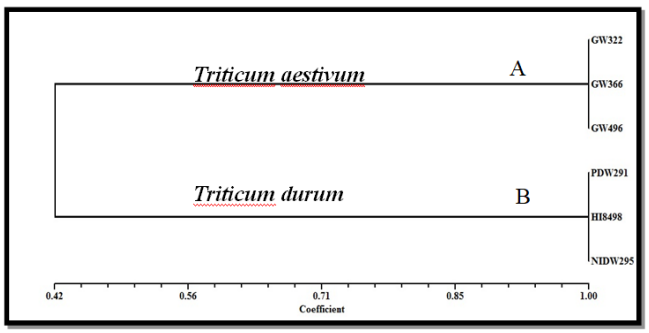


Figure 6. Dendogram of SDS PAGE profile of total protein in different wheat varieties.

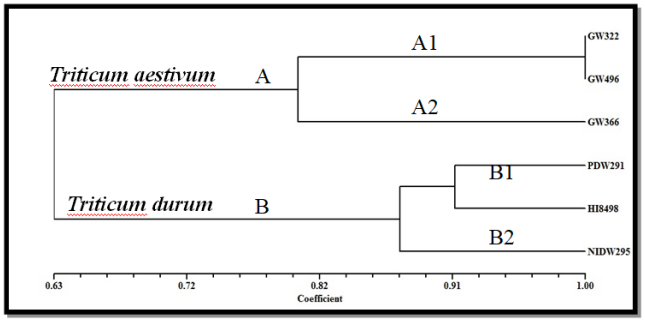


Figure 7. Dendogram of SDS PAGE profile of protein fraction albumin in different wheat varieties.

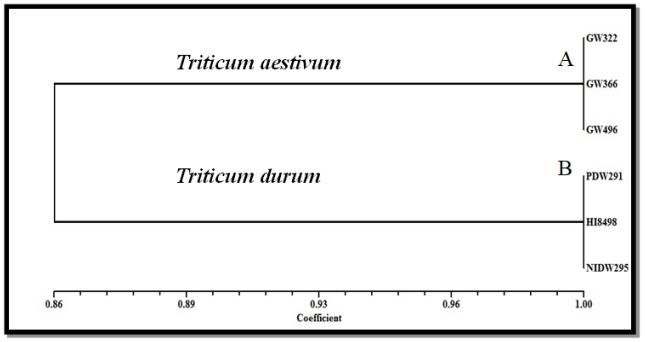


Figure 8. Dendogram of SDS PAGE profile of protein fraction globulin in different wheat varieties.

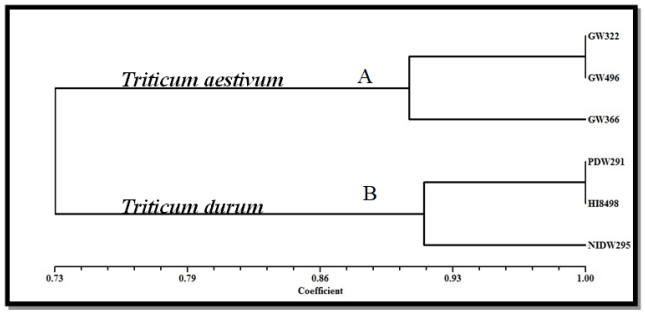


Figure 9. Dendogram of SDS PAGE profile of protein fraction gliadin in different wheat varieties.

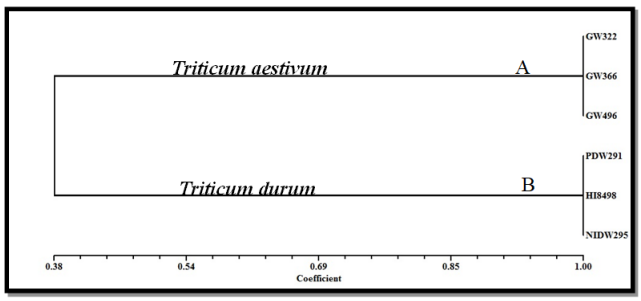


Figure 10. Dendogram of SDS PAGE profile of protein fraction glutenin in different wheat varieties.

4. Discussion

The SDS-PAGE analysis demonstrated its utility in identifying genetic diversity and distinguishing wheat cultivars based on protein banding patterns. The presence of 13 polymorphic bands and their distribution between *T. aestivum* and *T. durum* varieties underscores the molecular differences between these wheat species. Polymorphism within the molecular weight range of 50 to 150 kDa was particularly significant, supporting its value for cultivar differentiation[19].

Albumin fractions revealed unique bands specific to certain cultivars, such as GW 366 and NIDW 295, highlighting their potential as markers for varietal identification. The formation of subclusters in the dendrogram further corroborates the genetic distinction between *T. aestivum* and *T. durum* and also, described albumin proteins as potential biomarkers for wheat breeding[15,20]. Globulin fractions exhibited fewer polymorphisms, but the unique presence of band 8 in *T. aestivum* and its absence in *T. durum* reinforces the role of globulin proteins in distinguishing between these species[10,21].

Gliadin fractions showed clear differentiation between *T. aestivum* and *T. durum* in which polymorphism in gliadin fractions were found across wheat soecies[2,22]. Bands 7, 9, and 11 serve as reliable markers for species-specific identification. The glutenin protein analysis highlighted the significance of HMW-GS and LMW-GS in determining dough quality. The Glu-D1 allele 5+10, linked to superior bread-making quality, was exclusive to *T. aestivum*, while the Glu-B1 allele 20+8, advantageous for pasta production, was found in *T. durum* emphasized the importance of glutenin subunit profiles in wheat quality assessment[23].

Overall, the electrophoretic patterns of total proteins and their fractions provide a robust framework for distinguishing between *T. aestivum* and *T. durum* wheat varieties, facilitating targeted breeding and quality assessment.

5. Conclusion

Electrophoresis of total seed proteins and their fractions proved to be a valuable technique for differentiating wheat varieties and understanding their genetic relationships. Specifically, the glutenin fraction demonstrated the presence of distinct alleles (5+10 and 20+8) that reliably characterized *T. aestivum* and *T. durum* wheat, respectively. These findings align with the known suitability of *T. aestivum* for bread making and *T. durum* for pasta production.

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7. Author Contribution

Mohammedtarik Saiyad and Saleha Diwan: Conceptualization; writing, review and editing N. J. Patel and J. G. Talati: supervision and review. All authors have read and agreed to the published version of the manuscript.

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