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A comparative study of protein profiles and quantities in different wheat genotypes using SDS-PAGE analysis

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Abstract
This study aims to compare the protein profiles and quantities of different wheat genotypes of *Triticum aestivum* and *Triticum durum* using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The researchers hypothesize that the protein profiles and quantities would differ among different genotypes and the differences may be associated with the end-use quality of the wheat. The study used seven genotypes of *T. aestivum* and eighteen genotypes of *T. durum* grown under normal conditions. The albumin-globulin extraction, gliadin extraction, and extraction of soluble glutenin were done to sequentially extract defatted wheat flour to study the protein profiles. The results of this study could provide valuable information for wheat breeders and researchers to develop new wheat cultivars with improved end-use quality.

Keywords: Wheat genotypes, protein profiles, SDS-PAGE analysis, albumin + globulin extraction, end-use quality

Introduction
Wheat is one of the most important cereal crops worldwide, with an annual production of over 700 million tons (FAOSTAT 2021) [3]. It is a vital source of dietary protein, carbohydrates, vitamins, and minerals for humans and animals. Among the various types of wheat, *Triticum aestivum* (common wheat) and *Triticum durum* (*durum* wheat) are the most widely cultivated and consumed species. Common wheat is primarily used for bread-making, while durum wheat is used for pasta and couscous production (Shewry and Hey 2015) [12]. The quality of wheat flour and its end products, such as bread and pasta, depends on the quantity and quality of wheat proteins. Gluten, a mixture of two major protein groups, glutenin and gliadin, is the primary determinant of wheat dough properties (Shewry and Hey 2015) [12]. The quality of gluten and its composition varies among different wheat genotypes and environmental conditions, such as temperature, rainfall, and soil fertility (Payne and Lawrence 1983) [8]. Therefore, the identification and quantification of wheat proteins are crucial for developing high-quality wheat cultivars for various end uses.
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a widely used method for separating and analyzing proteins based on their molecular weight (Laemmli 1970) [4]. SDS-PAGE analysis provides information on the quantity, molecular weight, and relative abundance of different protein bands in a sample (Coico *et al*. 2005) [1] It has been extensively used for studying the protein profiles of various plant species, including wheat (Shewry *et al*. 2010; Lv *et al*. 2021) [11, 7]. However, limited research has been conducted to compare the protein profiles and quantities of different wheat genotypes using SDS-PAGE analysis. In this study, we aimed to compare the protein profiles and quantities of different wheat genotypes of *T. aestivum* and *T. durum* using SDS-PAGE analysis. We hypothesize that the protein profiles and quantities would differ among different genotypes, and the differences may be associated with the end-use quality of the wheat.
Previous research has shown that the protein content and composition of wheat flour are affected by various factors, such as genotype, environment, and management practices (Shewry and Hey 2015; Lv *et al*. 2021) [12, 7]. Moreover, the protein content of wheat grains varies between 8% and 20% depending on the genotype and environmental conditions (Payne and Lawrence 1983). Similarly, the quantity and composition of gluten proteins differ among wheat genotypes (Shewry and Halford 2002) [10]. Additionally, the SDS-PAGE analysis has been used to study the protein profiles of different wheat genotypes and has provided valuable information on the differences in gluten protein composition (Shewry *et al*. 2010; Lv *et al*. 2021) [11, 7].

This study aims to compare the protein profiles and quantities of different wheat genotypes of *T. aestivum* and *T. durum* using SDS-PAGE analysis. The results of this study could provide valuable information for wheat breeders and researchers to develop new wheat cultivars with improved end-use quality.

Materials and Methods

For this study, seven genotypes of *T. aestivum* L. (TA) and eighteen genotypes of *T. durum* (TD) were used. The wheat genotypes were sown under normal conditions in a randomized block design (RBD) with two replications to observe the experimental material. Recommended agronomic practices and plant protection measures were followed to ensure the successful growth of the crops.

Albumin-Globulin Extraction: A minor modification of the Osborne procedure, as reported by Lookhart and Bean (1995) [6], was used to sequentially extract defatted wheat flour (0.5 g). The flour was extracted using a 0.5 M NaCl aqueous solution (10 mL). Extraction was achieved by repeated stirring for 30 minutes at 4 °C, followed by 15 minutes of centrifugation at 20,000 rpm. The supernatants (albumin and globulin (Alb and Glob)) were transferred to a volumetric flask, and 50 mL of 0.5 M NaCl was added. The mixture was vortexed in 10 mL of deionized water for 1 minute, centrifuged for 5 minutes, and the supernatants were removed. An additional wash with water was performed to reduce the impact of salt in the pellet on gliadin extraction.

Gliadin Extraction: Globulin was extracted with 10 mL of 70% ethanol by incubating at 4 °C for 30 minutes followed by centrifugation at 20,000 rpm for 15 minutes. The gliadin supernatant (extracted three times) was transferred to another flask and made up to 50 mL with 70% ethanol.

Extraction of Soluble Glutenin: Similar methods were used to extract glutenins (Glu) from the gliadin pellet in three stages using 7 mL of 50% 1-propanol and 1% dithiothreitol (DTT). The volumetric flask was filled with 25 mL of extraction solution and mixed with the Glu-1, Glu-2, and Glu-3 extracts. Majorly glutenin extracts are made up of high and low molecular weight (HMW & LMW) glutenin subunits (Verejiken *et al*. 2000) [14].

SDS-PAGE Gel Electrophoresis: To analyze the wheat protein, sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12% separating gels and 5% stacking gels in a vertical electrophoretic setup, following the method of Laemmli (1970) [3]. The protein was diluted in a 1:2 (v/v) ratio with the sample buffer consisting of 0.055 M Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS), 20% glycerol, 4.3% mercaptoethanol, and 0.0025% bromophenol blue. The protein was then heated at 90 °C for 2 minutes and cooled to room temperature. Electrophoresis was performed on an 11% gel at 60 V for 4 hours, followed by staining with Coomassie Brilliant Blue R-250 stain (0.05%) for 2 hours, destaining, and documentation. The protein molecular weight markers were used to estimate the molecular weights of the subunits.

Result and Discussion

Wheat is an important cereal crop that provides a major source of dietary protein. It contains several classes of storage proteins, such as albumins, globulins, gliadins, and glutenins, which are involved in the development of gluten. In the present study, we evaluated the content of different storage proteins in 25 wheat genotypes using a combination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and extraction profiling of storage protein. The results are presented in Table 1, the electropherogram in Figure 1 showed that each wheat variety had a unique set of protein bands, and the resolution of the protein region between 20 and 14 kDa was insufficient to identify several components separately. The study found that -gliadin components were composed of one to three polypeptides with molecular weights of 55, 60, and 65 kDa (Lew *et al*. 1992) [5].

Albumin content was found to range from 38.7±1.1 (HI8498*MACS4028) to 48.5±1.0 (DDW 47) percent of total protein. The highest albumin content was observed in DDW 47 genotype, whereas the lowest was observed in HI8498*MACS4028. A similar study also reported a wide variation in glutenin content among wheat genotypes (Wieser *et al*., 2023) [15]. Albumins are water-soluble proteins that are rich in essential amino acids, and they play a crucial role in seed germination and seedling growth (Shewry and Halford 2002) [10]. The soluble glutelin content varied from 10.2±1.3 (HI8498*MACS4028) to 18.1±1.5 (DDW 47). The highest soluble glutelin content was found in DDW 47 genotype, whereas the lowest was observed in HI8498*MACS4028. Soluble glutelins are also important seed storage proteins, and they contribute to the elasticity of dough, a key factor in determining the quality of wheat flour (Shewry and Halford 2002) [10].

The insoluble glutelin content ranged from 21.2±1.2 (HI8713*HI8498) to 32.7±0.8 (DDW 47). The highest insoluble glutelin content was found in DDW 47 genotype, whereas the lowest was observed in HI8713*HI8498. Insoluble glutelins are important in determining the strength and elasticity of dough and are known to be associated with gluten quality (Shewry and Halford 2002) [10]. Gliadin content varied from 20.2±2.1 (GW322) to 27.1±1.8 (MP3382). The highest gliadin content was observed in MP3382, whereas the lowest was observed in GW322. Gliadins are known to be the major allergenic components of gluten and are responsible for triggering celiac disease in susceptible individuals (Sapone *et al*. 2012) [9]. However, a high gliadin content in the grain can result in poor nutritional value of the flour due to the low levels of lysine, tryptophan, and sulfur-containing amino acids found in gliadins. Therefore, better knowledge and understanding of the various physicochemical properties, protein quality, polymorphism of the various protein fractions, and information on the amino acid composition of the varieties will be beneficial not only to plant breeders but also to processors for the manufacturing of food products without affecting their nutritional value (Siddiqi *et al*. 2020) [13].

Table 1: Protein content and gliadin subunit concentration in grains of different wheat genotypes (% of total proteins)

S.N.	Genotypes	Albumin + Globulin	Soluble Glutelin	Insoluble Glutelin	Gliadin	γ + β + α gliadins (Rich subunit)	ω-gliadins (Poor subunit)
1	GW322 (TA)	40.2±1.1	14.4±1.8	22.3±0.9	20.2±2.1	42.3±1.5	1.2±0.4
2	HI1634 (TA)	43.4±1.2	15.2±1.0	28.5±0.8	22.3±1.9	51.2±1.3	1.4±0.6
3	MP3382 (TA)	46.1±1.2	13.4±1.5	24.6±1.1	27.1±1.8	48.4±2.0	0.92±0.4
4	JW1203*MP3382(TA)	39.3±1.1	12.4±1.4	27.2±1.4	22.3±1.7	44.5±1.8	1.1±0.8
5	JW1203*HI1634(TA)	41.2±1.3	11.6±1.2	23.4±0.9	24.5±1.9	42.1±1.2	1.4±0.2
6	WB02*GW322(TA)	40.7±1.6	12.5±1.3	22.5±0.7	21.4±2.1	45.6±0.9	1.4±0.4
7	HI1633*MP3382(TA)	39.6±1.4	14.5±1.1	23.6±1.1	22.8±2.3	47.2±1.1	0.98±0.8
8	HI8713(TD)	41.2±1.5	15.6±1.4	27.3±1.3	24.5±2.1	43.2±1.7	1.4±0.5
9	MACS 4028(TD)	42.4±1.2	17.2±1.3	29.4±1.2	22.3±1.8	45.1±1.7	1.2±0.9
10	DDW 47(TD)	48.5±1.0	18.1±1.5	32.7±0.8	23.5±1.9	42.4±1.5	1.4±0.9
11	HI8498*HI8759(TD)	43.5±1.3	13.2±1.6	22.3±0.3	23.4±1.8	41.4±1.3	1.3±0.9
12	DDW47*MACS4028(TD)	44.5±1.4	14.2±1.7	24.5±1.3	22.2±1.9	44.3±1.3	1.3±0.5
13	HI8713*HI8759(TD)	46.2±1.4	15.6±1.8	29.5±1.4	24.5±2.1	45.6±1.4	1.4±0.7
14	HI8713*MACS4028(TD)	41.5±1.3	17.2±1.9	25.6±1.5	22.1±2.2	46.8±1.4	1.5±0.8
15	HI8713*HI8498(TD)	43.6±1.2	12.3±1.2	21.2±1.2	20.4±2.3	47.2±1.7	1.2±0.6
16	HI8498*MACS4028(TD)	38.7±1.1	10.2±1.3	23.4±1.3	24.5±1.9	43.4±1.9	0.96±0.8
17	MACS4028*DDW47(TD)	45.2±1.4	16.2±1.1	29.6±1.4	24.3±1.8	42.1±1.8	0.99±0.7
18	HI8759*HI8498(TD)	44.4±1.3	12.4±1.3	24.5±1.3	21.5±2.1	45.1±1.5	1.1±0.4
19	HI8759*HI8713(TD)	41.6±1.4	15.6±1.4	22.4±1.1	23.5±2.3	46.2±1.7	1.3±0.5
20	MACS4028*HI8713(TD)	45.2±1.2	12.4±1.7	23.6±1.2	24.9±2.2	43.2±1.6	1.3±0.8
21	HI8498*HI8713(TD)	40.8±1.3	12.3±1.4	28.3±0.7	22.5±1.8	41.6±1.5	1.4±0.5
22	MACS4028*HI8498(TD)	45.2±1.3	14.2±1.7	29.2±0.8	21.1±1.9	44.5±1.2	1.2±0.6
23	DDW47*HI8759(TD)	43.1±1.1	12.3±1.3	27.3±0.9	24.5±2.0	46.7±1.4	1.6±0.7
24	HI8498(TD)	40.6±1.4	13.4±1.1	27.1±1.0	25.6±2.1	42.3±1.6	1.3±0.9
25	HI8759(TD)	40.7±1.3	14.3±1.2	22.3±1.3	21.1±2.2	42.6±1.4	1.1±0.7

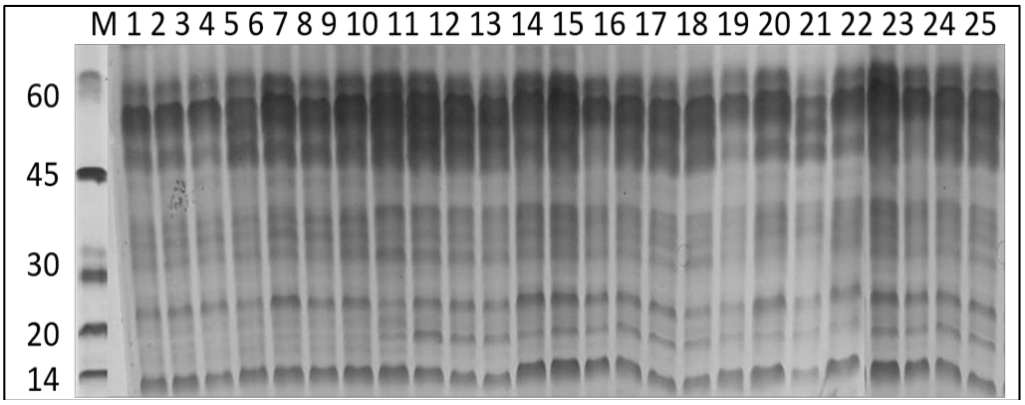

Fig 1: SDS-PAGE patterns of different wheat genotypes showing protein distribution (M- Protein marker in Kda)

The content of γ + β + α gliadins, which are the rich subunit of gluten, ranged from 41.4±1.3 (HI8498*HI8759) to 51.2±1.3 (HI1634). The highest content of γ + β + α gliadins was found in HI1634, whereas the lowest was observed in HI8498*HI8759. The γ + β + α gliadins are important in determining the viscoelastic properties of gluten, which contribute to the quality of bread dough (Shewry and Halford 2002) [9]. The lowest content of ω- gliadins was observed in MP3382, whereas the highest was found in DDW47*HI8759. Omega-gliadins typically exhibit higher acidity compared to numerous α- and γ-gliadins, falling within the molecular weight range of 46 to 66 kDa in 2-Dimensional Electrophoresis (2-DE) (Cho K *et al.*, 2018) [2].

Conclusion

The present study showed that there is significant variation in the content of different storage proteins among wheat genotypes, which could have important implications for the quality and nutritional properties of wheat products. Further studies are needed to explore the relationship between storage protein content and gluten quality and to identify genotypes with desirable protein profiles for specific end uses.

Authors Contribution

Conceptualization of research and designing of the experiments (RSS and VV); Execution of field/ lab experiments and data collection (VV); Analysis of data and interpretation (VV); Preparation and editing of the manuscript (RSS and VV).

Conflict of Interest

The authors declare no conflicts of interest.

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