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COMPARATIVE ANALYSIS OF DIFFERENT FRACTIONS AND SDS-PAGE PROTEIN PROFILES IN MUTANT AND NON MUTANT WHEAT (*TRITICUM AESTIVUM L.)*VARIETIES

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Article Info

Abstract

In Pakistan **different** wheat varieties are grown by mutation breeding, or cross breeding, the most important breeding methods successfully used in domestic wheat to obtain new lines of mutant, cross breed or hybrid varieties which have better agronomic values. The current study was aimed to evaluate the mutation induced changes in different mutant varieties, cross breed and hybrid variety present in Sindh, Pakistan. Comparative study of 30 (10 mutant, 10 cross breed and 10 hybrid) wheat cultivars collected from NIA, (Nuclear Institute of Agriculture Tando jam) was conducted. Total protein, fractions of proteins and SDS- PAGE profiling of protein" were carried out at the laboratories of Institute of Biochemistry University of Sindh Jamshoro. All experiment were performed by applying standard analytical methods and data were examined using the T test and one-way analysis of variance (single factor) ANOVA to determine whether a value was significant ($P < 0.05$) or very significant ($P < 0.001$). The findings showed that the application of induced mutation has a great impact on varieties development and their properties which are significantly differed. The Albumin content exhibited significant differences among varieties while the total protein constituent observed constant. These finding suggests that mutation breeding and genetic background of crossbreed or hybrid varieties may influence nutrient profile of wheat varieties. However, the data of this research study will be an important resource, in breeding strategies for growing wheat varieties with desired composition, nutritional and functional properties.

Keywords:
Wheat, mutant varieties, biochemical composition, protein fractions, phenolic compounds, nutritional quality

Introduction

The value of the wheat nutrition is very important as it takes an important place between the various crop species being extensively produced as staple food of Pakistan [1]. The wheat significance is mainly due to the fact that it can be ground into flour, which form the basic ingredients of wheat products thus it considers the key nutrients source of the most of the Pakistani citizenries [2]. Wheat's affordability, adaptability, and nutritional richness make it a cornerstone of the Pakistani diet, especially for the economically disadvantaged sections of the population that is way it holds paramount significance in the Pakistani diet, particularly for the impoverished segments of the population. Wheat flour widely used in the preparation of many food products such as bread, chapatti, noodles and pasta [3] A big portion of people consumes fewer calories than the recommended standard, increasing malnutrition. Limited choices in the dietary habits, especially among the poor, contributing micronutrient deficiencies [4,5]. Wheat is a good source of nutrient for calories due to its rich composition of carbohydrate and protein contribute in growth and physiological functions, however the nutritional and biochemical composition of wheat varieties depends on breeding methods, climate and soil conditions. [5] Wheat protein are mainly classified as soluble storage protein (Albumun, Globulin) and insoluble Gluten as functional protein (gliadin & Glutenin) associated with viscoelastic properties of wheat dough and overall nutritional composition of the wheat varieties. The protein content of these fractions present in wheat flour play an important role in human nutrition [6]. The viscoelastic properties of wheat gluten also make it suitable for many wheat products such as chapatti, wheat bread, noodles and pasta [7].

Moreover, many approaches have been applied for genetic improvements such as mutation breeding, hybridization, chromosome engineering and DNA markers for the development of herbicide resistant varieties with improved nutritional qualities. [2, 8, 9]. Most of the mutant wheat varieties have been developed through induced mutation by radiations such as gamma rays(called radiation induced mutation for large scale chromosomal rearrangement) and chemical mutagenesis such as by ethyl Metha sulfonate (EMS) cause point mutation by altering the DNA sequences [10].

Hybridization and conventional breeding are also important fundamental breeding method to develop new varieties by creating novel genetic variations, contributing improved characteristics and nutritional traits [11, 12] In regard to the protein composition, it is agreed that the total protein content is relatively constant among wheat cultivars [13, 14] however, there is a great deal of variation in the content of certain fractions of the protein, such as the albumin and globulin, which have been attributed to the mutagenesis or breeding process [15-17]. Glutenin and gliadin fractions were always identified as important factors in dough extensibility and bread-making quality and were found to vary due to genetic and environmental differences [18, 19]. Also, it was reported that the nitrogen fertilization can modulate the content of glutenin and gliadin which affect functional properties [20, 21]. The current study was designed to comparatively evaluate and screening of protein fractions of mutant and non-mutant wheat varieties. The results of this study will contribute in the development of nutritionally improved varieties and will support in future wheat breeding programs aimed at improving food quality and human health.

MATERIAL AND METHODS

This research involved the collection of a total of 30 wheat varieties, including 7 mutant varieties and 3 varieties that had a mutant parent, from the Nuclear Institute of Agriculture in Tando jam (NIA) as shown in Table 1 were compeered to non-mutant (crossbreed & hybrid) (n=20) wheat varieties

TABLE 1 THE NAME OF WHEAT VARIETIES WITH BASIC INFORMATION

Mutant Varieties		Mutagen Treatment
1.	Kiran -95	Chemical/NaN3, 4Hours
2.	Sohat-90	Chemical/NaN3
3.	Johar-78	Physical/fN, 6Gy
4.	Johar-18	--------
5.	Tatara	
6.	Bakhtawer-92	Physical/gamma rays
7.	WL-711	Mutant Line

PROCESSING AND PREPARATION OF WHEAT SAMPLE

Preparation of flour sample: The husk was removed from the wheat grain and ground into fine flour which was then passed through a 100-sieve mesh. Fine flour samples were stored in air tight containers for further processing.

extraction of the different protein fraction from wheat flour

Extraction of different protein fraction was carried out by the method of Ibtessam Tahir Ansari *et al,* 2021 with slight modification. 500mg of wheat flour was suspended in 1000µL of various extraction buffers overnight to allow for optimal solubilization, and then sonicated for a duration of 30 minutes. The resulting suspension was then subsequently clarified through centrifugation at 14000 revolutions per minute (4°C) for 20 minutes, and the clear transparent supernatants obtained were utilized for quantification of protein (Albumin) water-soluble proteins were extracted using water, while salt-soluble proteins (globulin) were extracted using 0.15N NaCl. Gliadin was extracted using Tris-50% isopropanol buffer, and glutelin was extracted using 0.1M NaOH. For additional examination, the resultant supernatant was kept at-40°C [23].

quantification of protein

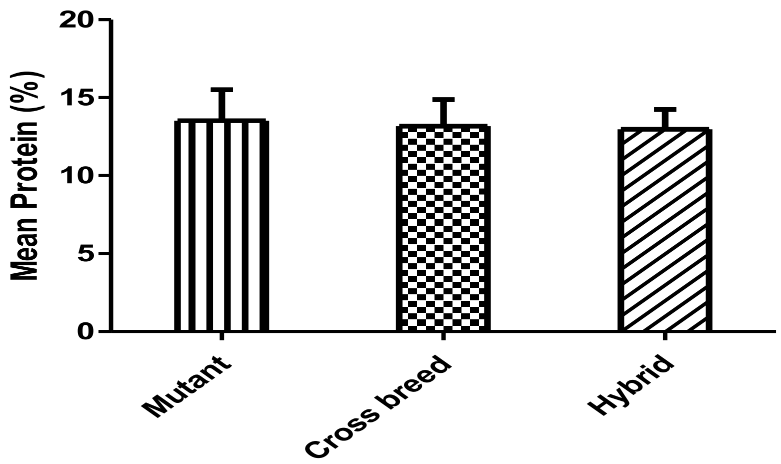
To determine the protein content of the sample extract, the Bradford test was performed using bovine serum albumin (BSA) standard curves with linearity ranges of r=0.99 were used. The analysis was conducted in five replicates. For protein concentrations 5mL of Bradford reagent was added in 1mL of sample extract and mixture was incubated for ten minutes at room temperature. At 595 nm, the absorbance was measured and a calibration curve was prepared by plotting the absorbance versus protein amount in µg using the equation for a straight line obtained from the BSA standard curve (Bradford's assay). [23].

SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDSPAGE)

To screen out different protein fractions in mutant and non- mutant wheat varieties, SDS-PAGE was implemented under reducing conditions on 10% polyacrylamide separating and 4.5%. stacking gel following the method described by Ibtessam et al. 2011 [24]. Phosphate buffer with 0.1M (pH 7.4) was used to extract the 20µL of wheat sample and 5µL of pre-stained protein marker "Thermo Fisher Scientific" was applied to each individual well and subjected to electrophoresis at a consistent current of 30 mA/gel for an approximate duration of 2 hrs at a temperature of 20 °C (Mini slab gel, Bio-Rad Laboratories, Hercules, CA, USA). Subsequently, the gels underwent staining with a solution containing 0.25% Coomassie brilliant blue (CBB) G-250 mixed with 2-propanol: acetic acid & Water at a ratio of 5:1:5, followed by immersion in 30% methanol and 10% acetic acid until the background accomplished transparency and the protein fractions manifested as distinct blue-hued dark and light bands. Bio-Rad Gel documentation system was used to captured Gel images to provide the molecular weight of each protein fraction [24].

RESULTS AND DISCUSSIONS

The total protein (%) content of mutant, cross breed and hybrid wheat varieties is illustrated in Figure 1. No significant differences were observed in percentage of total protein content within three cultivars. The results of the present study are supported by Singh *et al* 2001 [25] investigations that reveals total protein content (%) in mutant wheat varieties did not significantly differ from non-mutant (crossbreed and hybrid) wheat varieties. This suggests the probability of genetic stability, similarity in metabolic pathways, or balanced experimental conditions as potential explanations for the uniformity in protein content.

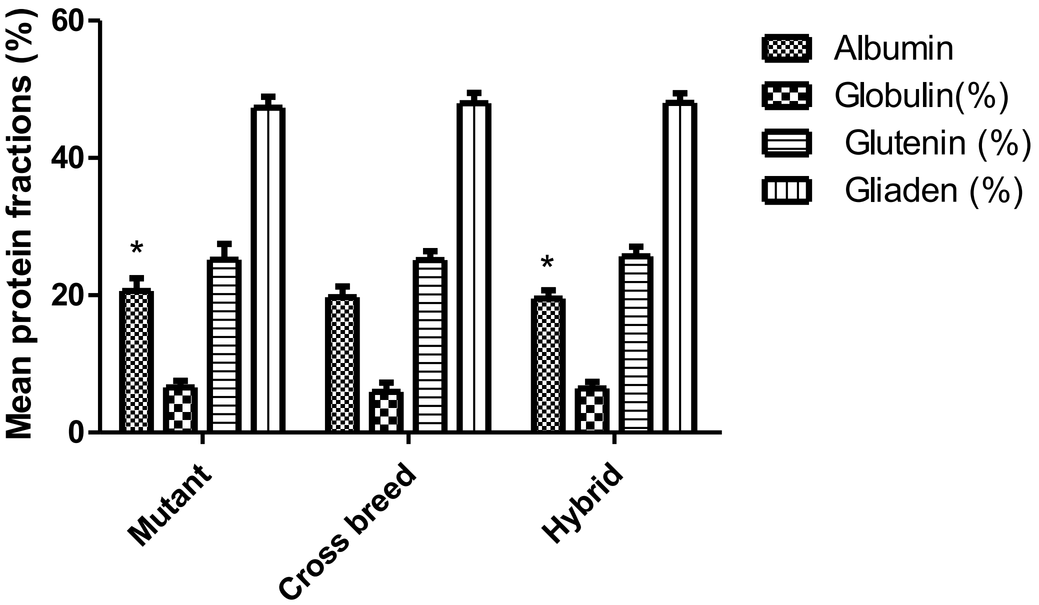


Mutant (n=10), Cross Breed (n=10) Hybrid (n=10), *P< 0.05, ** P< 0.01, ***P< 0.001

Figure 1 Determination of Protein (%) content in mutant and non -mutant wheat varieties

There were no statistically significant differences in the total protein content of the three varieties of wheat (mutants, hybrids, and crosses) indicate uniformity in protein constituents among varieties.

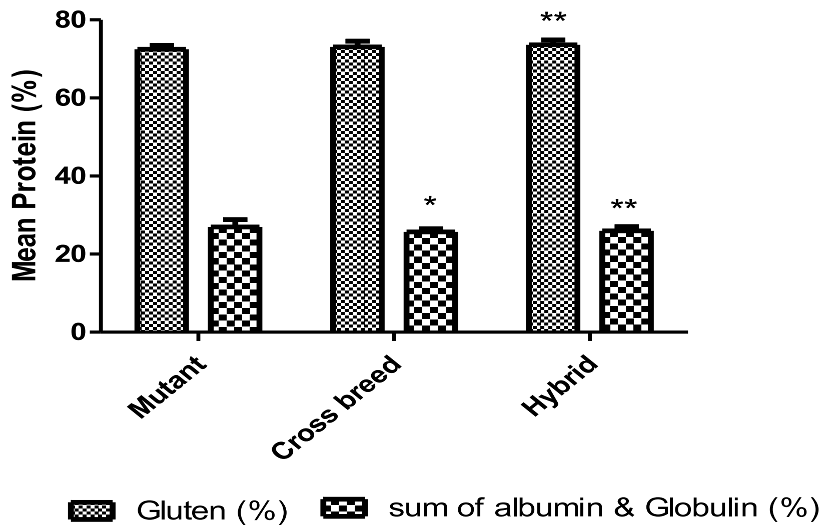
Many studies in term of total protein demonstrate the genetic stability in different wheat cultivars including those produced by mutagenesis may be caused by consistency in breeding methods may frequently possess analogous metabolic pathways regarding protein synthesis. Researchers also indicates environmental factors may play significant role in varied protein levels, but in controlled laboratory atmosphere, for initial breeding settings protein content may not have varied significantly. [26]



Mutant (n=10), Cross Breed (n=10) Hybrid (n=10), *P< 0.05, ** P< 0.01, ***P< 0.001

Figure 2 Determination of different protein (%) fractions in Mutant and non- Mutant varieties

Figure 2 demonstrate the (%) value of different protein fractions of mutant, and non-mutants (cross breed and hybrid) wheat varieties. Investigation of the (%) distribution of different protein fractions in mutant, crossbreed, and hybrid wheat varieties, show a significant (*P<0.05). difference in total albumin as compared to hybrid while, other soluble and insoluble fractions of protein do not exhibit any significant variations. which may emphasize the need of targeted breeding methods. Many researchers discussed the role of protein (albumin) in wheat cooking and baking quality. They also demonstrate constancy of certain protein fractions despite genetic modifications in mutant and non-mutant wheat [27, 28].. The findings were further supported by Unbehend et al. (2003),whose carried out detailed estimation of wheat protein structures and revealed that different protein fractions including both soluble and insoluble fractions were observed stable among different wheat varieties, supporting the results of current study with no significant differences in these fractions [29]. A review of the available literature provides additional insight into the observed differences. Zhang et al. (2021) determine the effects of low temperatures on the protein fractions of wheat, thus helps in the understanding of environmental factors effect on grain structure, wheat plant growth, protein structure and percent composition [30].



Mutant (n=10), Cross Breed (n=10) Hybrid (n=10), *P< 0.05, ** P< 0.01, ***P< 0.001

Figure 3 Comparison of total soluble fractions Albumin and Globulin with sum of insoluble fractions glutenin and gliaden as gluten among three cultivars.

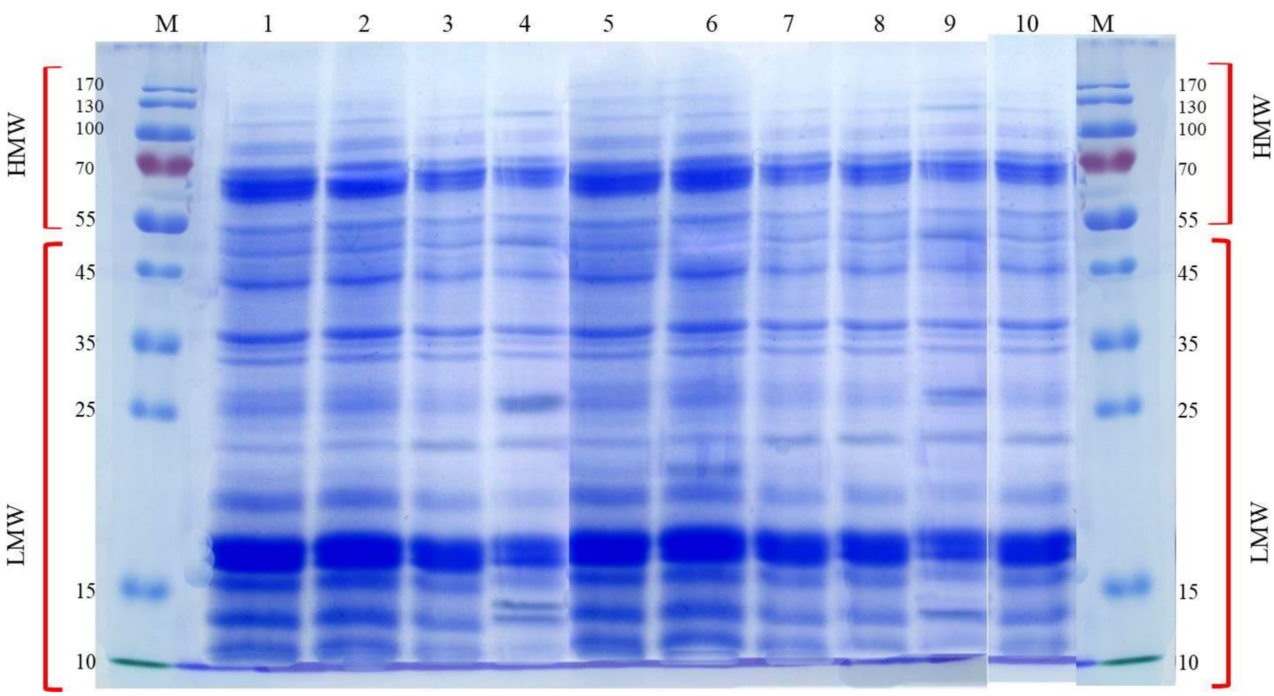
This study provides a complete comparison of the total soluble fractions ("albumin and globulin") and total insoluble fractions ("glutenin and gliadins"), collectively referred to as gluten in non-mutant, Crossbreed and Hybrid wheat cultivars. Notably, significant differences in total insoluble Fraction (sum of the glutenin and gliaden =gluten) were observed in mutant and Hybrid varieties but not in Crossbreed varieties. Meanwhile, the total soluble fraction (combination of albumin and globulin) in Mutant wheat varieties, was significantly different from both non-mutant Crossbreed and Hybrid wheat varieties. One-way analysis of variance (ANOVA) confirmed the total soluble fractions statistically significant ($P<0.05$) and showed even higher significance ($P<0.01$) for total insoluble fractions (sum of glutenin and gliaden) as gluten. The significant results of the determination of protein fractions provide valuable information and are important for managing breeding strategies, applied processing methods, aimed to developed new cultivars with additional nutritional and functional properties

Although the total protein content among the mutant and non-mutant wheat varieties remained relatively constant, significant variations were observed in individual protein fractions, particularly albumin, globulin, gliadin, and glutenin. The differences in albumin content may indicate compensatory changes in other storage protein fractions such as globulins or gluten proteins. Since wheat proteins exist as a complex mixture of metabolic and storage proteins, an increase or decrease in one fraction is often balanced by changes in another fraction to maintain overall nitrogen equilibrium within the grain.

The altered distribution of protein fractions may also influence the functional and technological quality of wheat flour. Albumins and globulins are mainly associated with enzymatic and metabolic functions, whereas gliadin and glutenin are the principal gluten-forming proteins responsible for dough rheology. Variations in gliadin-to-glutenin ratio can directly affect dough extensibility, elasticity, and baking performance. Increased glutenin fractions generally contribute to stronger dough structure through the formation of intermolecular disulfide bonds, while higher gliadin levels improve dough viscosity and extensibility. Therefore, the observed protein fraction variability among mutant wheat lines suggests that mutagenesis may influence flour quality and end-use characteristics in addition to nutritional composition.

The SDS-PAGE profiles Further supported these findings by revealing differences in low molecular weight proteins (10–55 kDa), corresponding mainly to albumins, globulins, and gliadin subunits, and high molecular weight proteins (55–170 kDa), which primarily represent glutenin polymers. Mutant wheat varieties exhibiting stronger high molecular weight bands may possess improved gluten network formation and enhanced baking quality compared to non-mutant varieties.

SCREEING OF AND IDENTIFICATION OF DIFFERENT FRACTIONS OF PROTEIN THROUGH SDS-PAGE ELECTROPHORESIS

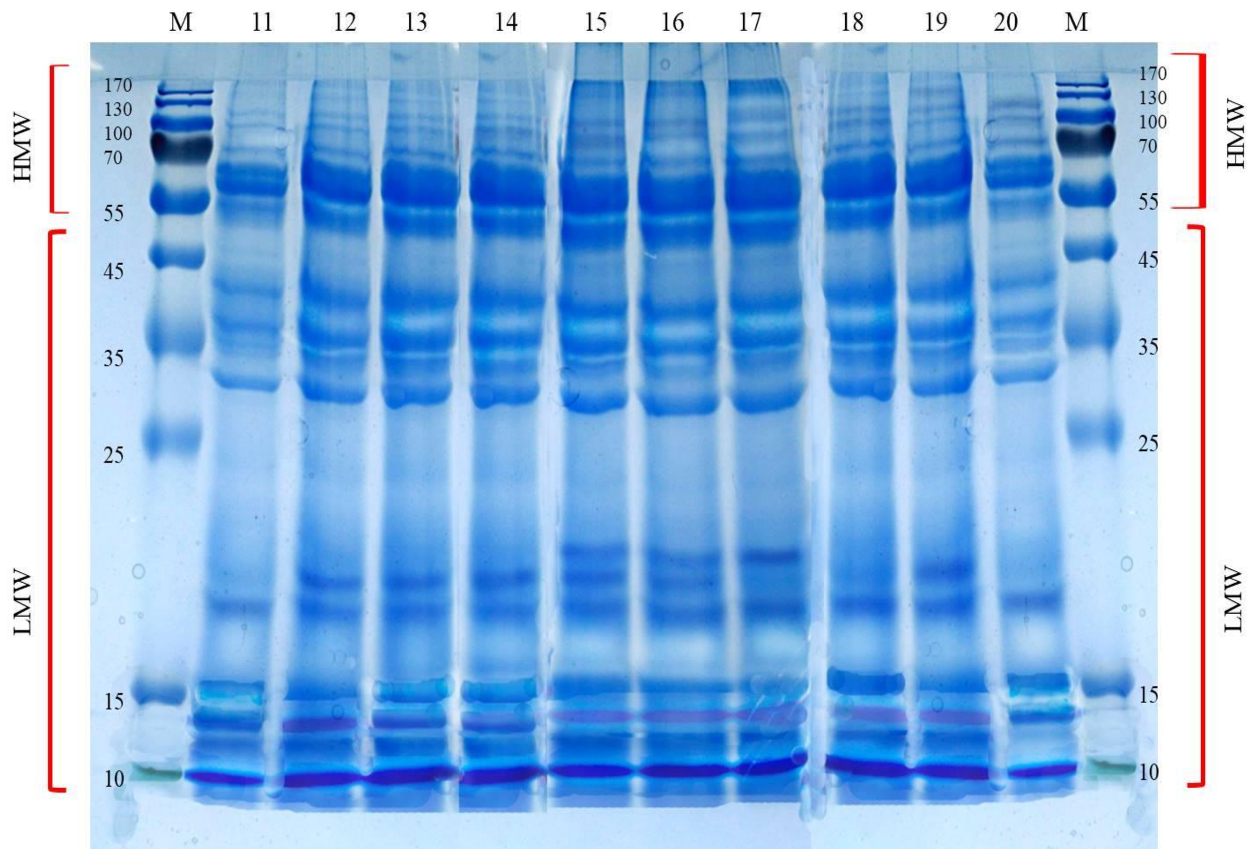


M= protein marker (10 to 170 kDa), 1-10 mutant wheat varieties

Figure 4 Screening of protein fractions in mutant wheat varieties

TABLE 2 TOTAL NUMBER OF LOW MOLECULAR WEIGHT PROTEIN FRACTIONS AND HIGH MOLECULAR WEIGHT PROTEIN FRACTIONS OF MUTANT WHEAT VARIETIES

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10
HMW Protein Fractions	5	6	4	4	5	5	4	4	6	4
LMW Protein Fractions	13	12	12	11	14	11	12	12	9	12

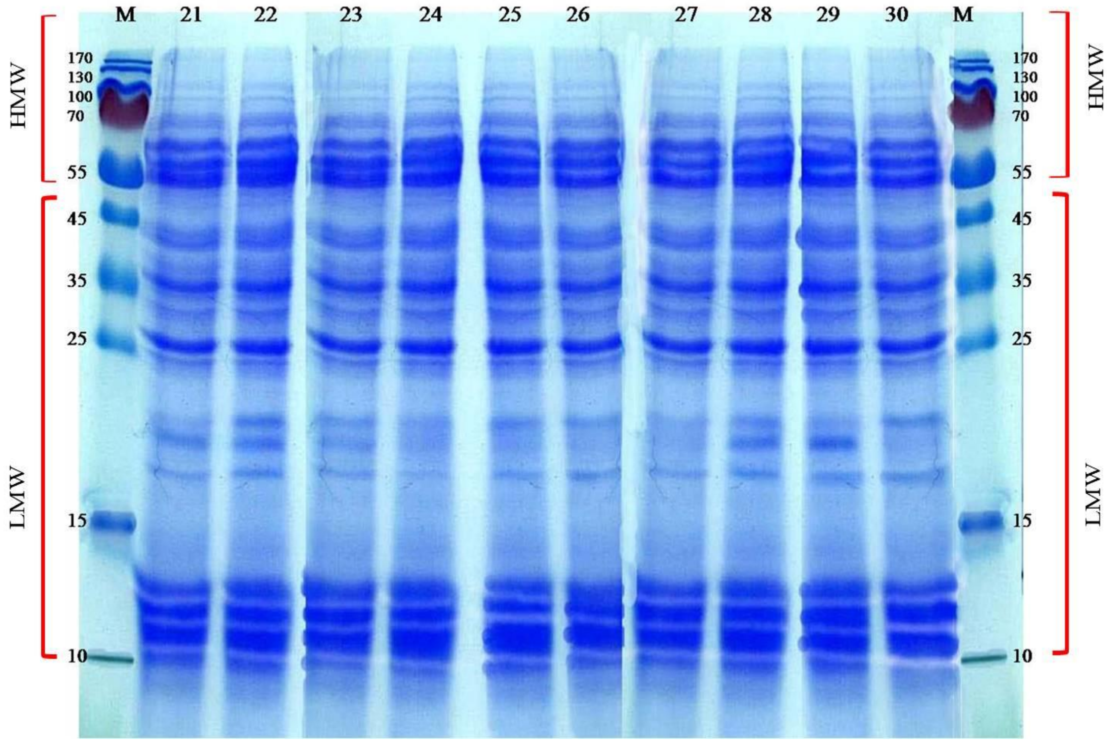


M= protein marker (10 to 170 kDa), 11-20 mutant wheat varieties

Figure 5 Screening of protein fractions in non-mutant wheat varieties

TABLE 3 TOTAL NUMBER OF LOW MOLECULAR WEIGHT PROTEIN FRACTIONS AND HIGH MOLECULAR WEIGHT PROTEIN FRACTIONS OF NON- MUTANT WHEAT VARIETIES

	Lane 11	Lane 12	Lane 13	Lane 14	Lane 15	Lane 16	Lane 17	Lane 18	Lane 19	Lane 20
HMW Protein Fractions	5	5	5	6	5	4	6	5	6	5
LMW Protein Fractions	9	10	10	11	11	12	11	9	11	8



M= protein marker (10 to 170 kDa), 21-30 mutant wheat varieties

Figure 6 Screening of Protein Fractions in Non-Mutant Wheat Varieties

TABLE 4 TOTAL NUMBER OF LOW MOLECULAR WEIGHT PROTEIN FRACTIONS AND HIGH MOLECULAR WEIGHT PROTEIN FRACTIONS OF NON-MUTANT WHEAT VARIETIES

	Lane 21	Lane 22	Lane 23	Lane 24	Lane 25	Lane 26	Lane 27	Lane 28	Lane 29	Lane 30
HMW Protein Fractions	6	6	4	6	6	6	6	6	6	6
LMW Protein Fractions	12	13	12	11	11	10	11	12	11	11

Screening of protein fractions through SDS-PAGE shows distinct variations in the pattern of protein fractions as well as the total number of protein bands in mutant and non-mutant wheat varieties. The low molecular protein fraction on SDS-PAGE gel (ranges from 10 to 55 kDa) primarily constitute soluble" Albumin , Globulin" metabolic protein which are involved in the and enzymatic and physiologic functions in the wheat grain while gliadin also a monomeric low molecular weight protein, (a fraction of gluten) contribute dough extensibility and viscosity in wheat products. Variations in the number of low molecular weight protein fractions and intensity of the band on SDS-GAGE gel show the differences in these protein within three types of selected varieties. The differences in band intensity observed on SDS-PAGE gel suggest that that the process of mutagenesis may alter the peptide bonding pattern and side chain interactions of protein

In contrast the high molecular weight protein fraction (Ranges 55-170 kDa) on SDS-PAGE represent the high molecular weight glutenin fraction (HMW-GS) of gluten protein which are polymeric and responsible for dough elasticity and baking quality of the wheat flour. These high molecular weight glutenin fractions rich in glutamine and proline shows more organized structure. Mutant varieties show variations in the number of high molecular weight protein and the low intensity of band on SDS-PAGE gel may indicate the consistency in total glutenin content observed in mutant and non-mutant wheat cultivar but stronger structural organization in gluten polymers. Screening of the wheat protein fractions clarified the protein profile and their structural pattern suggest the genetically modification of mutant wheat varieties influence the total protein pattern including low and high molecular weight proteins that determine the technological, nutritional, physiological and functional properties of wheat grain

CONCLUSION

This research is mainly focused on comparative determination of soluble and insoluble fractions and molecular weight profiles of protein among mutant and non- mutant wheat varieties. Although, determination of individual fractions of protein exhibit significant variations including albumin, globulin, gliaden, glutenin in mutant and hybrid wheat but the sum of these fractions (total protein) remain stable shows the mutagenesis influence individual fractions. Mutant varieties exhibiting higher albumin and globulin content suggest the enhanced nutritional properties while constant level of gluten fraction is crucial to maintain their functional and processing properties. This consistency may be attributed to genetic stability/shared metabolic pathways & controlled experimental conditions. These investigations contribute comprehensive information regarding complexity of protein and its composition in different wheat and will be beneficial future research framework, breeding strategies and the production of genetically improved wheat cultivars. This study outlines several directions for future research, including ongoing monitoring of crop production efficiency, molecular and genetic studies of mutant varieties and collaboration with local farmers, to evaluate the feasibility of introducing “mutant varieties” into local agricultural farming system.

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