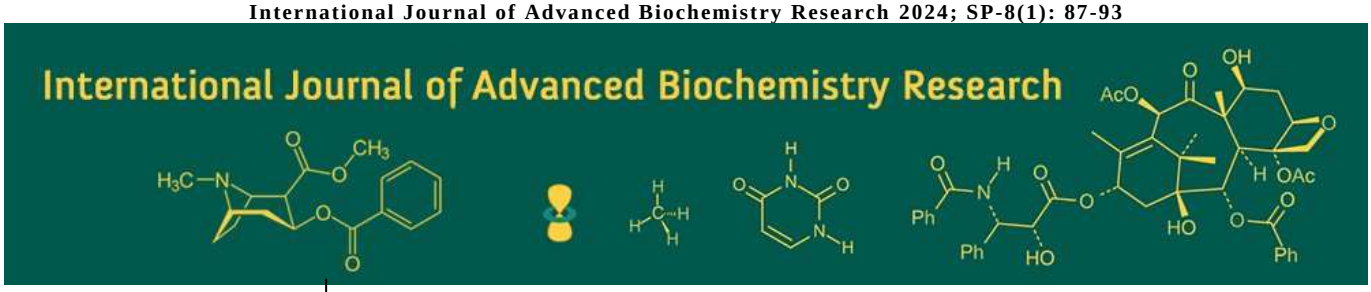

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HR Sodhaparmar
Young Professional-I, Bioscience Research Centre, Sardar Krushinagar Agricultural University, Gujarat, India

NJ Patel
Associate Professor, Department of Biochemistry, Anand Agricultural University, Anand, Gujarat, India

TJ Bedse
Biochemist, Regional Agricultural Research Station, Karjat, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (Dr. BSKKV), Dapoli, Dist. Ratnagiri, Maharashtra, India

Corresponding Author:
HR Sodhaparmar
Young Professional-I, Bioscience Research Centre, Sardar Krushinagar Agricultural University, Gujarat, India

Biochemical characterization of wheat and their anti-nutritional composition

HR Sodhaparmar, NJ Patel and TJ Bedse

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Abstract
Wheat (*Triticum* spp.) is an important cereal food and occupies a significant position among the cultivated cereals. It is the main source of the world's food energy and nutrition; more than 60% of the total daily requirements of protein and calories is met through wheat. The present investigation was carried out to evaluate the nutritional and antinutritional properties of bread and durum wheat (*Triticum* spp.). For the flour often wheat cultivars were analyzed, five from each bread and durum wheat species. The crude protein ranged from 10.27 to 13.41%, which showed higher value in durum cultivar. The total soluble sugar was varied from 1.62-2.92%. Which was higher in cultivar GW-496. The tryptophan and lysine varied from 2.12—3.90% and 0.78-1.06%, respectively. The wet and dry gluten was ranged from 16.98-27.91%, 5.90-16.84%. The β-carotene was ranged from 3.52-5.90 ppm. Phytic acid, the heat-stable anti-nutritional factor, it was ranged from 1.92mg/gm to 2.92 mg/gm. Trypsin inhibitor was ranged from 234.56 to 333.06 TIU. Result of the observed sedimentation value was varied between 29.17-47.01%. The same cultivars are used for genetic diversity study based on gluten fractions i.e. glutenin and gliadin on SDS-PAGE. Result reveled that glutenin protein fraction showed 35 bands of diverse molecular weight ranged from 14.4-150 kDa while, gliadin protein fraction showed bands of 30-80 kDa with unique ɣ-45 band in cultivar GDW-3, A-206 and GW-1. The ability of wheat flour to be processed into different foods is largely determined by its nutritional quality. Therefore, the assessing this relative importance of nutritional quality and human health, this study will be helpful in crop improvement.

Keywords: Nutritional, anti-nutritional factors, lysin, tryptophan, sedimentation value, glutenin, β-carotene

Introduction
Wheat (*Triticum* spp.) is the staple food for millions of people around the globe as it is an important part of the daily requirement of the diet and main source of world's food energy and nutrition. The most widely grown is common wheat (*T. aestivum* L.). It is a hexaploidy (AABBDD) with the chromosome number of 42 (2n = 6x = 42) and a genome size of 16 GB. A *T. durum* Desf. is a tetraploid species with two diploid genomes AA and BB. Each of these genomes has seven pairs of chromosomes (n =14 and 2n = 28). Following China, India holds the position of the world's second-largest wheat producer. In the 2021-22 timeframe, India made a significant contribution of 107.74 million tons to global wheat production. Gujarat, a significant western state, cultivates a substantial quantity of wheat. Gujarat accounted for 3.25% of the total area and 2.87% of the total production of wheat in the country (Anonymous, 2021-22) [4]. Wheat is good source of carbohydrates and other important nutrients including proteins, fiber, minor components including lipids, vitamins, minerals, and phytochemicals, which ultimately determines a quality of end product. Most (78-85%) endosperm protein of wheat is gluten, a very large complex primarily comprised of polymeric (Multiple polypeptide chains linked by disulfide bonds) and monomeric (single-chain polypeptides) proteins known as glutenin and gliadins, respectively. Due to extensive polymorphism, that gluten fraction; glutenin and gliadin have been widely used for cultivar identification in hexaploidy and tetraploid wheat (Shewry *et al.*, 2002) [34]. Therefore, assessing this relative importance of nutritional quality and human health, this study will be helpful in crop improvement.

Materials and Methods

Plant material: Five of each *T. aestivum* LOK-1, GW-322, GW-366, GW-451, GW-496 and *T. durum* A-206, HI-8498, GW-1255, GDW-3, GW-1 were received from Regional Research Station, AAU, Anand Agricultural University, Anand. Wheat flour was used for glutenin-gliadin fractionation on SDS-PAGE and for various qualitative parameters as well as antinutritional parameters.

Qualitative parameters

Moisture content from wheat flour was determined using the method described by AOAC. (1995) [3]. Ash content was determined using the method described by AOAC. (1965) [2]. Total soluble sugars were determined using phenol sulphuric acid method described by Dubois *et al.* 1956 [10]. The method of AOAC. (1965) [2] was used for determination of protein content. Lysine was determined using method described by Bhatnagar *et al.* (2007) [6]. Tryptophan content was determined using method described by Nurit *et al.* (2009) [23]. Total polyphenol content was analyzed by method described by Singh & Jambunathan, (1981) [35]. β-Carotene was determined using method described by Mishra and Gupta, (1998) [21]. TKW were determined by using method of AAAC. (2000) [1]. Iron and zinc were determined by Atomic adsorption spectroscopy by as per the procedure described by Lindsey and Norvell, (1978) [18]. Wet gluten, dry gluten, and gluten index were determined using the method described by AACC. (2000) [1].

Antinutritional parameters

Phytic acid was determined by using method described by Makkar *et al.* (1993) [19]. Trypsin inhibitor was analyzed by Hammerstrand *et al.* (1981) [13].

Protein analysis: Glutenin and gliadin protein fractions was extracted using protocol described by Pfiuger *et al.* (2001) [28]. Protein was then separated in sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE). Gel was then stained and destained as described by Sadasivam & Manickam, (1992) [33].

Data analysis: Qualitative and antinutritional parameters data obtained were analyzed using a completely randomized block design (CRD). The mean value for each of the quality attributes was based on analysis of three replicate samples. Analysis of variance (ANOVA), appropriate for the design, was carried out to determine the significance of differences among the cultivars for each of the parameter under study. Protein bands were scored for their presence (1) or absence (0) in each cultivar. Data entry was done in to a binary data matrix as discrete variables and analysis was done using NTSYSpc version (Rohlf, 1994) [32].

Results and Discussion

Qualitative parameters

The moisture percentage of all studied wheat cultivars was from 9.51 to 10.58%. According to Nasir *et al.* (2003) [22], 9 and 10% moisture content is suitable for storage stability and longer shelf life of wheat flour. The flour of HI-8498, GW-1, GW-322, GW-366 and GW-496 is suitable for storage stability and longer shelf life, whereas cultivars A-206, GW-1255, GDW-3 and LOK-1 showed the slightly higher range of moisture of 10.08 to 10.58%. Ash is primarily concentrated in the bran and is an indicator of flour yield (Prabhashankar *et al.*, 2002) [29]. In studied wheat cultivars which was found from 1.49 to 2.54%. Cultivars GW-322 and GW-451 showed highest ash 2.54% and 2.50% respectively. The Total soluble sugar content in flour was found to be varied from 1.62 to 2.92%. The cultivars GW-1 had the highest total soluble sugar content, whereas, GW-1255 was having the lowest percentage of total soluble sugar.

Table 1: Proximate composition present in wheat

Cultivar	Moisture (%)	Ash (%)	TSS (%)	Total protein (%)	Lys/ Protein (%)	Tryp/Protein (%)	β-carotene (ppm)	S-value (ml)	Total phenol (mg/gm)	TKW (gm)	Iron (ppm)	Zinc (ppm)
A -206	10.58	2.00	2.23	13.30	2.12	1.06	4.03	32.02	3.14	48.36	45.23	39.00
HI-8498	9.89	2.02	1.71	12.06	2.45	0.85	4.42	33.77	3.12	48.96	38.99	35.41
GW-1255	10.08	1.83	1.62	13.08	2.26	0.78	5.60	32.52	3.19	49.85	38.97	49.48
GDW-3	10.28	1.62	2.05	12.36	2.60	0.83	4.18	31.27	2.87	57.51	38.87	45.84
GW-1	9.70	1.49	2.92	13.41	2.42	0.89	4.08	29.27	2.77	59.12	47.37	38.68
LOK-1	10.14	2.31	2.66	11.93	2.97	0.81	3.89	38.52	2.56	39.26	38.89	34.80
GW-322	9.94	2.54	2.33	10.24	2.84	0.87	3.65	41.52	2.87	34.11	39.11	39.45
GW-366	9.51	2.13	2.59	11.28	3.85	0.86	3.59	47.02	2.41	47.58	38.87	28.28
GW-451	9.82	2.50	2.87	10.17	2.72	0.82	3.92	39.52	2.72	35.63	39.15	28.02
GW-496	9.73	2.52	2.82	11.84	3.90	0.77	3.52	44.02	2.42	36.88	39.09	37.16
S.Em.±	0.09	0.05	0.02	0.20	0.017	0.0121	0.10	0.65	0.05	0.52	0.455	0.436
C.D. at 5%	0.26	0.16	0.07	0.60	0.051	0.0359	0.29	1.92	0.15	1.54	1.343	1.287
C.V.%	1.51	4.7	1.95	2.96	1.083	2.466	4.23	3.05	3.10	1.98	1.95	2.01

The protein content of studied cultivars varied from 10.17% to 13.41%. Cultivars GW-1 and A-206, had the highest protein content of 13.41% and 13.30%, respectively while, GW-451 showed lowest value as 10.17%. The mean protein value of durum was found higher in durum cultivars (12.84%) than aestivum (11.09%) indicating grain hardness of durum is high. For making good quality pasta products from durum wheat, more than 12% is required, hence (Gupta *et al.*, 2002) [12] studied durum cultivars found to be good for pasta and bread making while, aestivum cultivars were good for bread and chapatti making.

The total amount of iron content of different wheat verities from ranged from 38.87-47.37 ppm. GW-1 showed significantly higher Iron content (47.37 ppm) followed by A-206 (45.23 ppm), whereas cultivar GW-366 has lowest 38.78 ppm zinc. The total amount of zinc content of different wheat verities was ranged from 28.02 ppm to 49.48 ppm. GW-1255 showed significantly higher zinc content (49.48 ppm) followed by GDW-3 (45.84 ppm), whereas cultivar GW-451 has lowest 28.08 ppm zinc. Result indicate that the Indian bread wheat or aestivum and pasta or durum wheat varieties possess low levels of grain iron (27-55 ppm)

and zinc (20-50 ppm). Therefore, there is a requirement of enhancing the iron, zinc and micronutrient content in wheat through biofortification (DWR, 2013) [11].

Lysine and tryptophan are limiting amino acids in wheat. Protein content is inversely related to lysine content in wheat, reported previous investigation (Jensen, 1976, *Brandt et al.,* 2000 and *Rharrabti et al.,* (2001) [15, 7, 32]. The lysine content in studied wheat cultivars varied from 2.12 to 3.90%, the mean value of lysine content was higher in aestivum cultivars (3.74%) than durum cultivars (2.37%) showed negative co-relation to the protein. Tryptophan content varied from 0.78 to 1.06%. Cultivar A-206 showed a highest value of tryptophan content (1.06%), while cultivar GW-496 showed lowest value of tryptophan content (0.77%). The polyphenol content of all the wheat varieties ranged from 2.41 mg/g to 3.19 mg/g. Cultivar GW-1255 shows significantly higher polyphenol content (3.19 mg/g), whereas cultivar GW-366 had a significantly lower value of polyphenol content (2.77 mg/g.). *T. durum* species showed higher polyphenol content as compare to *T. aestivum* showed their higher nutritive value of durum wheat than bread wheat.

Thousand-kernel weight (TKW) indicates about the grain quality i.e. longer, round and sound grains generally have higher TKW. TKW of all wheat cultivars ranged from 34.11 to 59.12 g. The mean TKW in all aestivum was 38.69 g, whereas in durum 52.76 g. From the result obtained, the mean value of durum cultivars was comparatively higher than that of aestivum cultivars due to bold and unshrivelled grain. Gluten, are major storage protein deposited in the starchy endosperm cells of the developing grain (Shewry *et al.,* 2002) [34]. Wet gluten is directly proportional to end-use quality (Kaushik *et al.,* 2013) [15]. Wet gluten, dry gluten, of flour was shown in the Table 2.0. Mean wet gluten and dry gluten of aestivum were 20.49% and 9.42% and of durum cultivars were, 25.44% and 14.37%.

Table 2: Wet gluten and dry gluten content of different *aestivum* and *durum* cultivars

Sr. No.	Cultivar	Wet gluten (%)	Dry gluten (%)
	T. durum		
1	A 206	27.60	16.52
2	HI-8498	24.27	13.20
3	GW-1255	23.44	12.37
4	GDW-3	24.02	12.95
5	GW-1	27.91	16.84
	Average	25.44	14.37
	T. aestivum		
6	LOK-1	23.67	12.59
7	GW-322	18.35	7.27
8	GW-366	21.07	10.00
9	GW-451	16.98	5.90
10	GW-496	22.42	11.34
	Average	20.49	9.42
	S.Em. ±	0.32	0.10
	C.D. at 5%	0.96	0.30
	C.V.%	2.45	1.50

β-carotene content ranged from 3.52 to 5.60 ppm. All aestivum cultivars ranged from 3.52 to 3.89 ppm, whereas all durum cultivars ranged from 4.08 to 5.6 ppm. Cultivar a GW-1255 showed higher β-carotene content among all wheat cultivars.

SDS-sedimentation volume was also positively correlated with puffing height of chapatti and negatively correlated with force required to compress the chapatti as assessed (Panghal *et al.*, 2017) [25]. The value of sedimentation found from 38.52 to 47.02 ml in aestivum cultivars and 29.17 to 33.77 ml in durum cultivars fig. 1.0 (Fig. 1.0). Our findings are in agreement with the values obtained by Patil *et al.* (2014) [26].

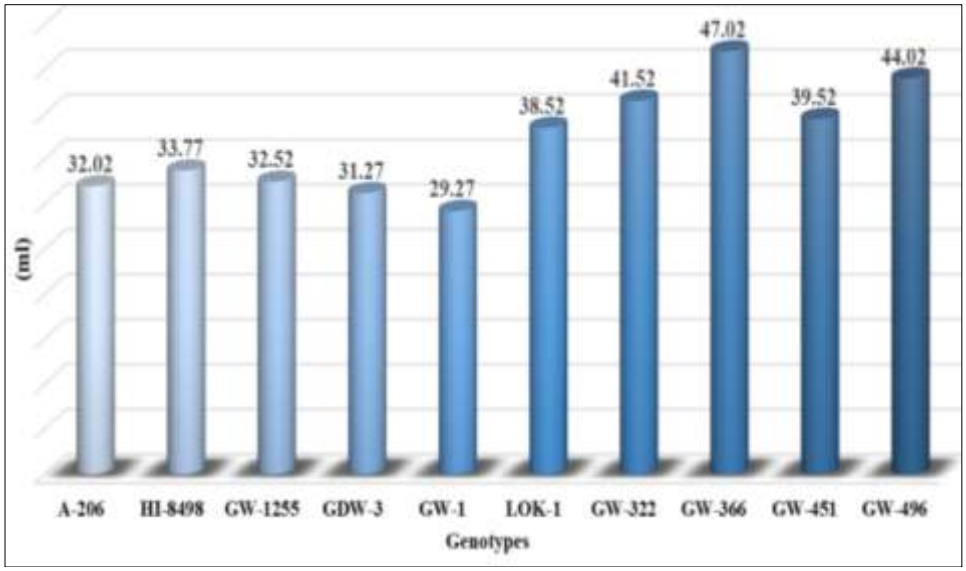

Fig 1: Sedimentation values of different wheat varieties of *T. durum* and *T. aestivum*

Sedimentation volume <20 ml is most suitable for the preparation of sweet biscuit/cookie/cakes (Baljeet *et al.,* 2017) [5]. Based on studied sedimentation values wheat cultivars are classified into three groups according to their suitability to the various product (Table 3.0).

Table 3: Classification of wheat cultivars for their suitable product type based on their sedimentation value

Group No.	Cultivars	Suitable product type
T. aestivum		
1	LOK-1, GW-322, GW-366, GW-451, GW-496	Chapatti (All purpose)
T. durum		
2	A-206, HI-8498, GDW-3, GW-1255	Chapatti
3	GW-1	Biscuit

Antinutritional factors

Phytic acid is known as a food inhibitor, which chelates micronutrient and prevents its bioavailability. In wheat caryopsis, it is present in bran fraction such as aleurone layer and pericarp. The limited bioavailability of cereals mineral content due to relatively low mineral levels and the presence of phytic acid and other antinutritional factors that reduce their bioavailability to 5–15% offers challenges in nutrition point of view (Das *et al.*, 2012) [10]. The Phytic acid content of all the wheat varieties ranged from 1.92 mg/g to 2.92 mg/g. Cultivar HI-8498 showed significantly higher phytic acid content (3.19 mg/g), whereas cultivar A-206 had a significantly lower value of phytic acid content (2.77 mg/g.). Phytic acid content was predominantly influenced by various factors like environment, genotype and growing locations, different edaphic factors and fertilizers applied (Reddy 2002; Kumar *et al.*, 2005; Steiner *et al.*, 2007 and Branković *et al.*, 2015) [20, 16, 37, 8].

Mikola and Kirsi [20] have reported that trypsin inhibitors also constitute 5 to 10% of water-soluble proteins in endosperm of wheat and other cereals like barley and wheat. Several studies indicate that trypsin inhibitor causes reduction in protein digestibility (Owusu *et al.*, 1970; Liener, 1976; Weerasooriya *et al.*, 2018) [24, 17, 36]. The trypsin inhibitor of all the wheat varieties ranged from 234.56 to 333.06 TIU.

Table 4: Composition of antinutritional factors present in different genotypes of wheat

Cultivar	Phytic acid (mg/gm)	Trypsin inhibitor (TIU)
A 206	1.92	333.06
HI-8498	2.92	287.52
GW-1255	2.15	246.20
GDW-3	2.40	234.56
GW-1	2.62	289.26
LOK-1	2.85	256.63
GW-322	2.76	340.62
GW-366	2.48	336.67
GW-451	2.41	249.99
GW-496	2.15	279.22
S.Em.±	0.038	2.74
C.D. at 5%	0.11	8.11
C.V.%	2.66	1.66

Electrophoresis of glutenin and gliadin protein fraction

Glutenin protein showed 35 bands of diverse molecular weight ranging from 14.4 to 150 kDa (Fig. 2.0). Though the varieties differed for the number of polypeptides, two main fractions of glutenin were observed in one-dimensional discontinuous gel that HMW-GS (45-150 kDa) and LMW-GS (14.4-45 kDa). The Jaccard's similarity index (SI) was recorded in order to evaluate the degree of closeness among varieties.

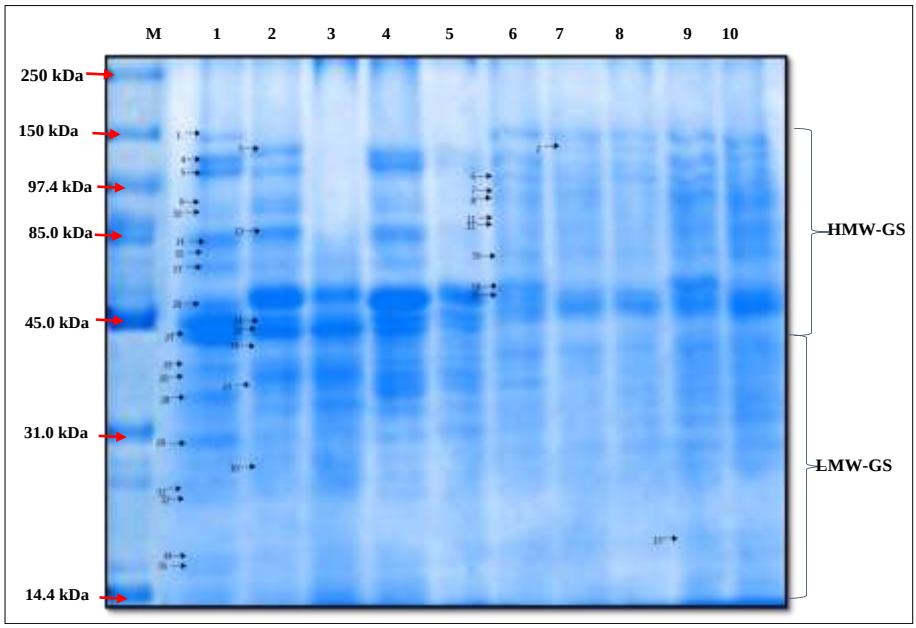

Fig 2: Banding pattern image profile of Glutenin protein fraction of different wheat varieties of *T. aestivum* and *T. durum*

1.	A-206	6.	LOK-1
2.	HI-8498	7.	GW-322
3.	GW-1255	8.	GW-366
4.	GDW-3	9.	GW-451
5.	GW-1	10.	GW-496

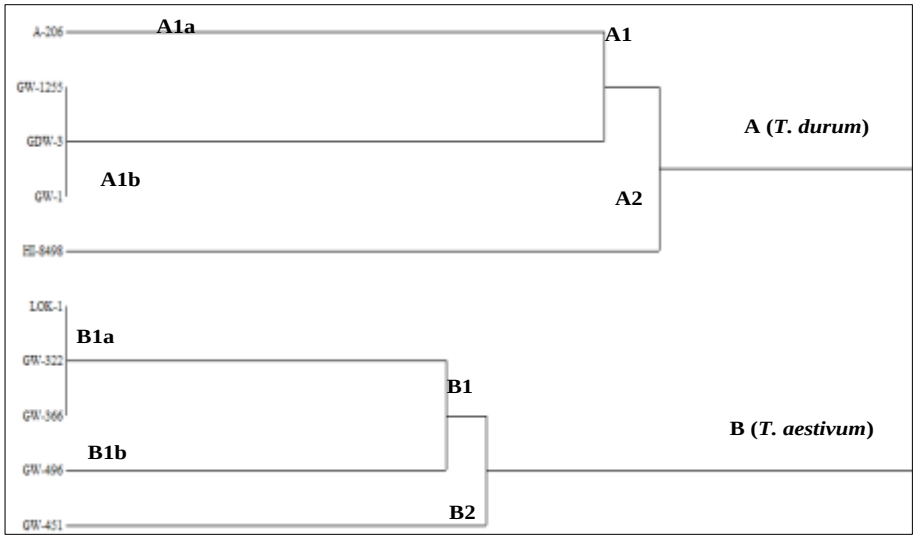

Fig 3: Dendogram of SDS-PAGE Profile of glutenin protein fraction in different *T. aestivum* and *T. durum* wheat varieties

Glutenin protein comprised two main clusters depicted as A and B. Main cluster A was comprised all durum wheat cultivars while, main cluster B was comprised all aestivum (Fig. 3.0). Main cluster A subdivided into sub-cluster A1 and A2. Sub-cluster A1 compromised only A-206 wheat cultivar. Subcluster A2 was again divided into A2a and A2b. Subcluster A2a was contained HI-8498 and GDW-3 whereas, subcluster A2b was comprised GW-1 wheat cultivar. Main sub cluster of *T. aestivum* (B) was divided into subcluster B1 and B2. Subcluster B1 was comprised two cultivars namely GW-322 and GW-366 wheat cultivars. Subcluster B2 was again divided into B2a and B2b. Among them subcluster B2a contains only LOK-1wheat cultivar while, sub cluster B2b contains GW-451 and GW-496 wheat cultivars.

The Gliadin protein was separated in α, β, γ and ω subunits ranging from 30-80 kDa on SDS-PAGE (Fig. 4.0). In durum wheat gliadin γ-42 and γ-45 serve as a marker for poor and good gluten strength, respectively (Payne *et al.*, 1984) [27]. The γ-45 was present in durum wheat cultivars A-206 GDW-3 and GW-1, which shows some specific characteristic in relation to end-use quality.

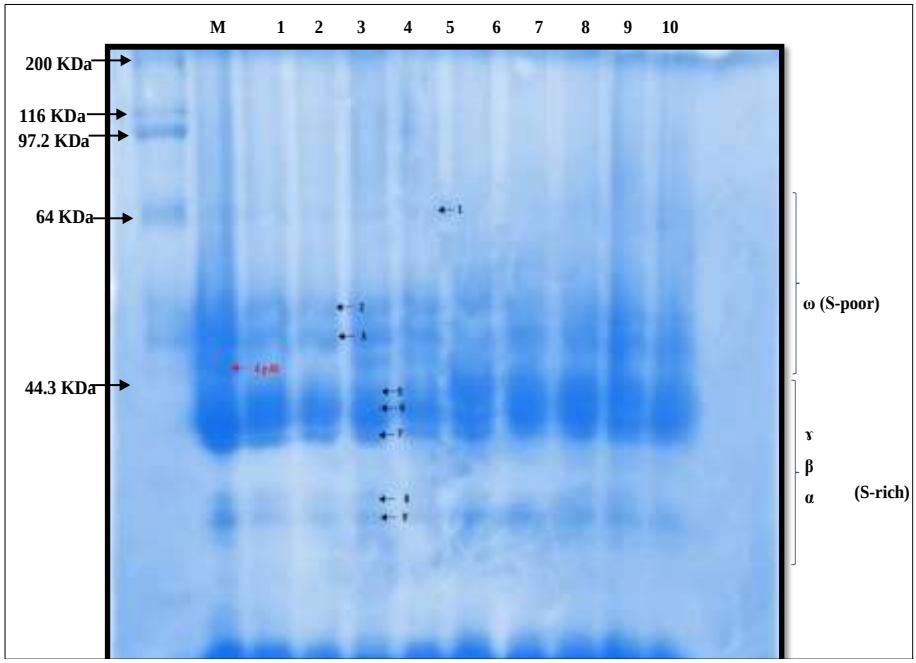

Fig 4: Banding pattern image of gliadin protein fraction on SDS PAGE of different wheat varieties of *T. aestivum* and *T. durum*

Glutenin and gliadin protein fractionation showed wide genetic variability and could be effectively useful marker for species identification based on variations in HMWGS and LMW-GS subunits banding pattern and intensity of bands [26].

Conclusion

Thus, it can be concluded that this study on proximate composition and anti-nutritional factor analysis will provides useful information in crop improvement strategies for wheat quality improvement through selection of germplasms with high quality traits with low phytic acid and trypsin inhibitor. The composition of gliadin and glutenin fractions largely cultivar specific and therefore should be considered for wheat baking quality assessment and breeding purposes i.e. marker for varietal and species identification.

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