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Electrophoretic Evaluation of Major Seed Storage Protein Fraction, Gliadins and Glutenins of Eighty-Six Indian Wheat Genotypes

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ABSTRACT

Wheat seed storage proteins, mainly gliadins and glutenins are the major proteins involved in the determination of bread-making quality. In this study, the variation in the polypeptide patterns of eighty-six Indian wheat genotypes and their interrelationships was studied using gliadins and glutenins fractions by using polyacrylamide gel electrophoresis. A substantial variation was recorded in gliadin fraction in the range of polypeptides of molecular weight range 40-45, 33-37, 29-32 kDa. The glutenins showed variation in the range of mol. wt. 75-110, 40-46, 22-36 kDa. Based on the polypeptide patterns of gliadin and glutenin fractions of eighty-six wheat lines, a dendrogram was constructed, which showed the genetic relationship among the different genotypes of wheat.

Key words: Dough quality, Fraction, Gliadin, Glutenin, Seed storage protein, Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.), is a cereal grain, formerly grown in the East of the Levant region but nowadays cultivated all over the world (Shewry, 2009). Due to its flour's food processing properties, particularly bread making, baked products, pasta and noodles etc. it has a major impact on human nutrition (Shewry, 2019). It is one of the most ancient domesticated crops that play an important role in crop species. This crop is extensively used as a food source and is also a central point for the commencement of agriculture (Khan *et al*. 2007). The seed storage proteins, glutenins and gliadins are the main constituents of gluten. These are further divided into α, β, γ-gliadins and the gliadins based on high and low molecular weight glutenins (HMW and LMW-Gs) (Nevo and Payne, 1987; Shewry, 1992). The baking quality in wheat flours depends on the amount of gluten present and the ratio of gliadins to glutenins (Cooke and Law, 1998; Uthayakumaran *et al*. 1999). The functional and rheological properties of wheat gluten and dough determined by the percent composition of gliadins and glutenins (Don *et al*. 2003). The seed storage proteins, gliadins and glutenins have also been extensively studied recently. The α-gliadins plays an important role in dough extensibility (Noma *et al*., 2019). They studied the association of the flour dough extensibility with the genes that encode three Gli-2 homoeoloci. Gluten is mainly composed of polymeric and monomeric proteins known as glutenins and gliadins, respectively (MacRitchie, 1999). Gliadins provide extensibility to the dough as it is viscous, whereas, glutenins provide elasticity to the dough (Payne *et al*. 1983; Nevo and Payne *et al*. 1987). Bietz and Wall (1972) divided glutenins into two types i.e. of low molecular weight glutenins (LMW Gs) (10-70 kDa) and high molecular weight glutenins (HMW Gs) (80-130 kDa) in wheat. The genetic variation in wheat has earlier been studied by using various types of markers like biochemical (storage proteins, antigen, antibody and hormone) and molecular markers (RAPD, SSLP, AFLP, VNTR, etc.). Figliuolo and Zeuli (2006) reported that RFLPs and protein storage markers were more explanatory for their capability to categorize germplasms. The variation in the glutenin and gliadin of the genotypes of durum wheat cultivars have recently been studied by Mefleh *et al*. (2019). They found a strong association with the glutenin than with gliadin content in the old wheat varieties. Moreover, the gliadins and glutenins fractions have been considered as much better marker system for wheat identification as the the protein profiling of albumins and globulins show slight differences in different wheat cultivars as compared to gliadins and glutenins (Dvoracek *et al*. 2001). In the present work, eighty-six Indian wheat varieties has been characterized based on the protein profiling of wheat seed protein fractions gliadins and glutenins on SDS-polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Plant material

Germplasm lines of wheat used in this study was kindly provided by the DWR (Directorate of Wheat Research) and CSSRI (Central Soil Salinity Research Institute).

Seed protein fractionation

The seed protein fractionation was carried out by using successively extraction method given by the Osborne with some modifications (Osborne, 1907). The flour was extracted with 0.5 M NaCl with intermittent vortexing for 1 minute every 10 minutes for 30 minutes. Then centrifuged the mixture for 10 minutes at 10,000 rpm at 4°C. The supernatant was collected and repeat this step three times. This supernatant contains albumin and globulin. The pellet now obtained, washed with distilled water to remove the residual salt. This residue was extracted with 70% aqueous ethanol for 30 minutes, with continuous vortexing for 30 minutes and centrifuged for 10 minutes at 10,000 rpm at 4°C. The supernatant was collected which contains gliadin. Glutenins were extracted from this pellet with 50% 1-propanol + 1% dithiothreitol for 30 min, with continuous vortexing for 30 minutes and centrifuged for 10 minutes at 10,000 rpm at 4°C. The supernatant was collected which contains glutenin. All these fractions were concentrated using acetone precipitation method. The protein residue obtained was left dried overnight and solubilized in protein extraction buffer.

SDS-PAGE electrophoresis

The SDS-PAGE was carried out on 10% polyacrylamide gels according to Laemmli with slight modifications (Laemmeli, 1970). After complete run gels were stained and destained. The protein bands were analyzed after complete destaining. The molecular weight of each and every protein band was calculated.

Data analysis

Based on the molecular weights of protein bands of gliadins and glutenins. A dendrogram was constructed from SPSS using a binary data matrix, which was calculated by the presence (1) or absence (0) of each protein band.

RESULTS AND DISCUSSION

Variations in the polypeptide patterns of gliadin and glutenin fractions

The banding pattern of gliadin and glutenin fraction of eighty-six wheat varieties was studied using SDS-PAGE, which shows that the molecular weight of gliadins ranged from 29 to 45 kDa (Fig 1) and molecular weight of glutenins was ranged from 22-110 kDa (Fig 2). The variation in the polypeptide patterns of gliadins and glutenins fraction was recorded in different molecular weight regions (Table 1 and Table 2, respectively). The gliadin fractions on the SDS-PAGE revealed the polypeptide variation in the three different molecular weight regions, i.e. 40-45 kDa, 33-37 kDa and 29-32 kDa (Table 1). Six different types of polypeptide variations were recorded in the mol. wt. region of 40-45 kDa of gliadin fractions. Whereas, in the range of mol. wt. 33-37 kDa and 29-32 kDa, only four different types of polypeptide patterns were recorded. In the case of glutenin fractions, polypeptides of mol. wt. regions 75-110 kDa, 40-66 kDa and 22-36 kDa showed large variations (Table 2). Based on the variation in the polypeptide patterns in these mol. wt. regions, eighty-six wheat genotypes were divided into eight, five and three groups, respectively.

Genetic relationship of wheat genotypes

The dendrograms were constructed using the genetic similarity coefficient matrix for gliadins and glutenins based on the presence and absence of the polypeptides on the SDS-PAGE (Fig 3 and 4). In dendrograms, eighty-six wheat genotypes were categorized according to their banding pattern on SDS-PAGE for both fractions. In both, the fractions dendrogram was divided into seven different clusters (Fig 3 and 4).

In the dendrogram of the gliadin fraction, cluster I included only two wheat lines HDR-77 and GW-396. The cluster II, III, IV, V, VI and VII comprises sixteen, thirteen, fifteen, nineteen, fourteen and seven wheat genotypes, respectively. The glutenin fraction was clustered the wheat genotypes into seven clusters in which the cluster I included thirteen wheat genotypes VL-738, HS-240, Kundan-DL-153-2, UP-2338, HI-977, Sonalika, HD-2329, WH-533, HD-2338, HD-2687, MACS-6145, Kalyansona and RAJ-3077. The cluster II, III, IV, V, VI and VII comprises nine, ten, fifteen, fourteen, fifteen and ten wheat genotypes respectively. The SDS-polyacrylamide gel electrophoresis is a very useful technique for effectively describing the genetic structure of crop germplasm (Ciaffi *et al*. 1993). Various studies have shown that baking quality of wheat dough and the presence of gliadin glutenins are interlinked (MacRitchie *et al*. 1992). Teng *et al*. (1988) analyzed that closely related wheat lines share identical banding patterns of gliadin proteins and could be used as genetic markers on the basis of their protein profiling on SDS-PAGE. Nizar (2002) also observed the same results for gliadin proteins and suggested to use it as a genetic marker. An, X *et al*. (2005) results show that on the basis of protein profiling of high-low molecular weight glutenins (HMW-Gs and LMW-Gs), wheat lines can be clustered in the dendrogram in distinct groups. He worked on 15 Iranian spelts, 25 bread and 270 European spelt wheat lines, which were studied by SDS-PAGE electrophoresis. Waines and Payne (1987) revealed that by increasing the expression of genes for HMW glutenins, bread wheat quality can be improved. So, it indicating the correlation between wheat quality and HMW glutenin. Tahir (2008) worked on wheat seed proteins HMW and LMW glutenins and constructed a dendrogram based on their protein bands. His findings concluded that for genetic diversity analysis, these protein profiles are very useful. Our findings agreed with Dvoracek and Curn (2003), who extracted four wheat

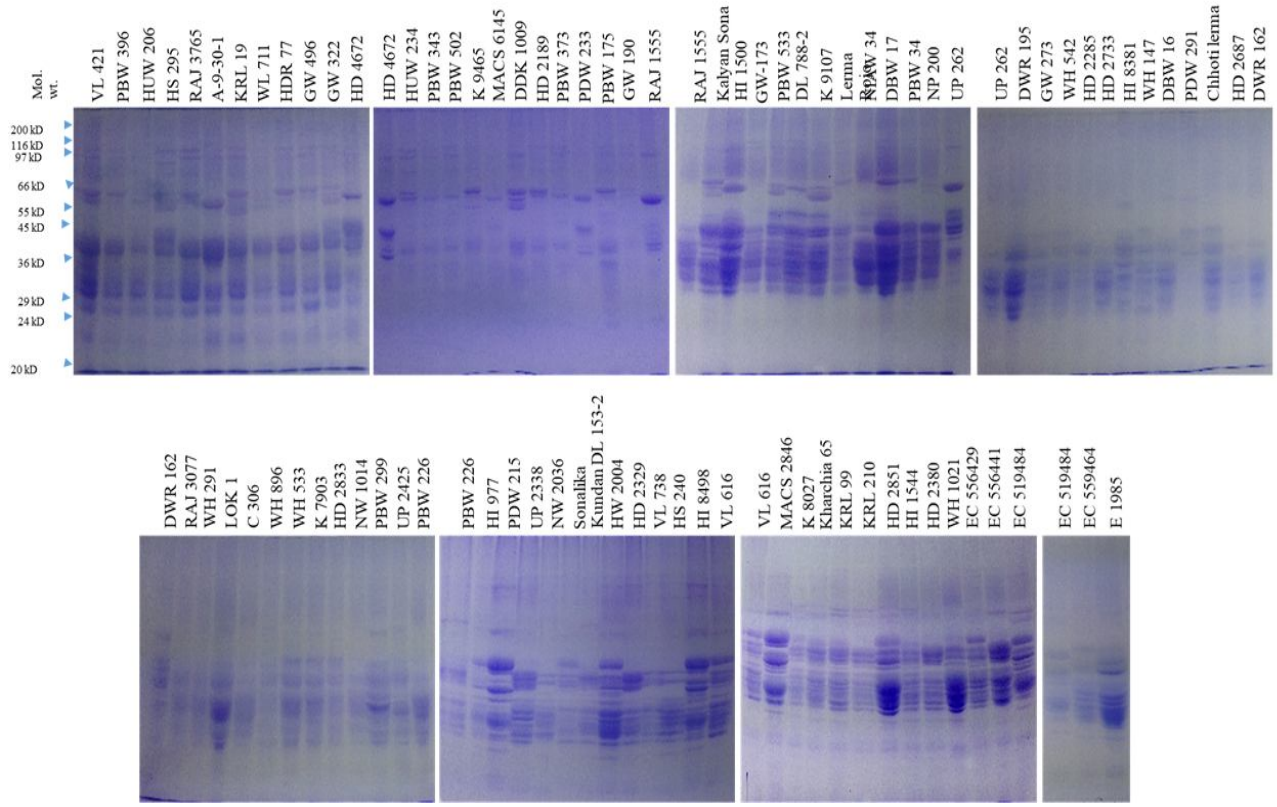


Fig 1: Variation in the polypeptide patterns of gliadin fractions of eighty-six wheat genotypes on SDS-PAGE.

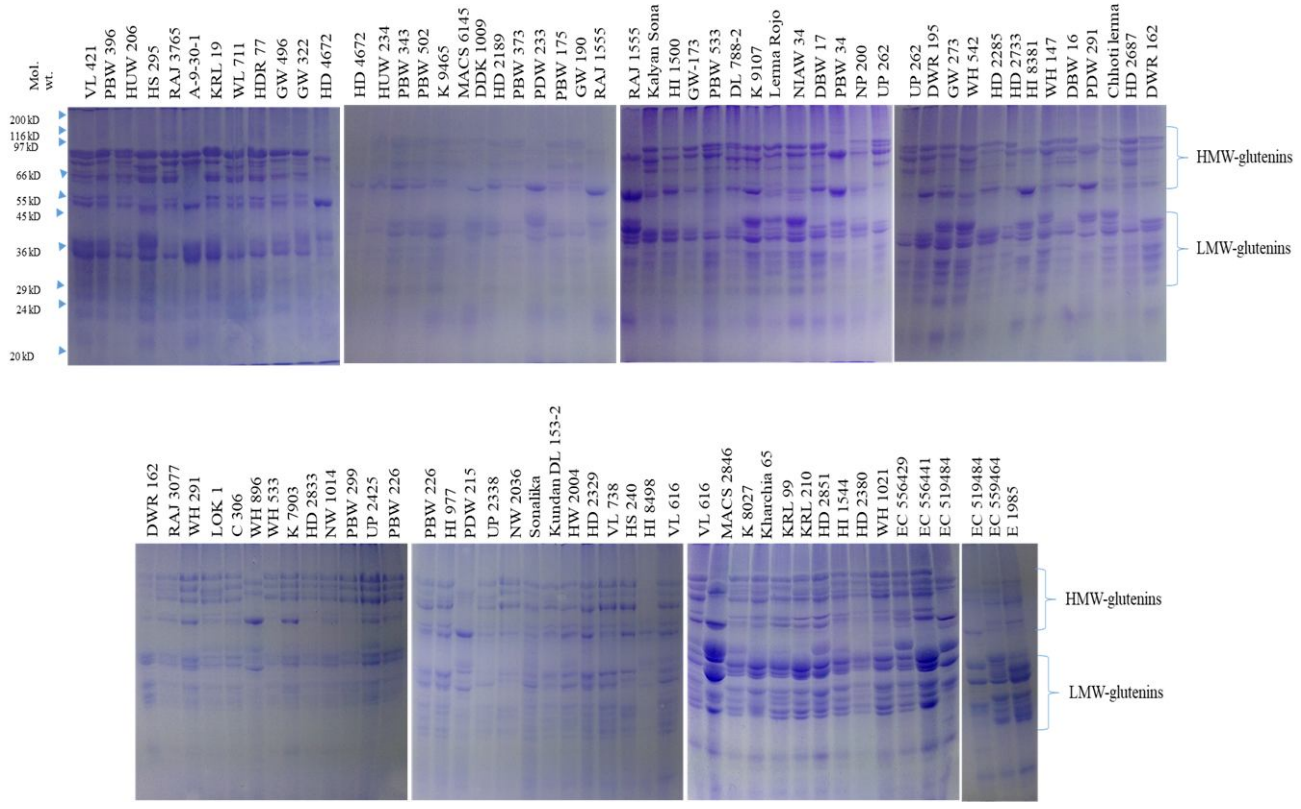


Fig 2: Variation in the polypeptide patterns of glutenin fractions of eighty-six wheat genotypes on SDS-PAGE.

Table 1: The variation in the polypeptide patterns of gliadin fraction of eighty-six Indian wheat genotypes on SDS-PAGE.

Molecular weight region	Molecular weights of polypeptides (kDa)	No. of lines	Wheat lines
40-45 kDa	40	25	HUW-206, WL-711, HDR-77, DDK-1009, PDW-233, HI-1500, DL-788-2, DBW-17, RAJ-3077, WH-291, C-306, WH-896, WH-533, K-7903, NW-1014, PBW-226, HI-977, UP-2338, NW-2036, Sonalika, Kundan-DL-153-2, HD-2329, VL-738, HS-240, WH-1021
	44	9	GW-322, RAJ-1555, GW-173, Lerma Rojo, NIAW-34, PBW-34, UP-262, HD-2380, EC-556441
	45, 40	14	GW-273, WH-542, HD-2285, HD-2733, HI-8381, DBW-16, HD-2687, LOK-1, PBW-299, UP-2425, PDW-215, HW-2004, MACS-2846, HD-2833
	43, 42	29	VL-421, PBW-396, HS-295, RAJ-3765, A-9-30-1, KRL-19, GW-496, HD-4672, HUW-234, PBW-343, PBW-502, K-9465, MACS-6145, HD-2189, PBW-373, PBW-175, GW-190, Kalyan Sona, PBW-533, K-9107, NP-200, DWR-195, Kharchia-65, KRL-99, KRL-210, HI-1544, EC-556429, EC-559464, E-1985
	45, 44, 40	6	WH 147, DWR 162, VL 616, K 8027, HD 2851, EC 519484
	45, 44, 42	3	PBW 291, Chhoti Lerma, HI 8498
33-37 kDa	35, 33	11	PBW-396, HS-295, RAJ-3765, A-9-30-1, HD-4672, HUW-234, PBW-502, PDW-233, PBW-291, EC-519484, EC-559464
	36, 35, 33	30	VL-421, HUW-206, WL-711, HDR-77, GW-496, GW-322, PBW-343, K-9465, MACS-6145, HD-2189, RAJ-1555, GW-173, PBW-533, Lerma Rojo, NIAW-34, DBW-17, WH-147, HD-2687, WH-291, LOK-1, WH-533, K-7903, HI-977, Sonalika, HD-2329, VL-616, HD-2851, WH-1021, EC-556441, EC-519484
	37, 35, 34	7	DDK-1009, PBW-373, PBW-175, GW 190, K-9107, DWR-162, PBW-299
	37, 35, 34, 33	38	KRL-19, Kalyan Sona, HI-1500, DL-788-2, PBW-34, NP-200, UP-262, DWR-195, GW-273, WH-542, HD-2285, HD-2733, HI 8381, DBW-16, Chhoti Lerma, RAJ-3077, C-306, HD-2833, NW-1014, UP-2425, PBW-226, PDW-215, UP-2338, NW-2036, Kundan-DL-153-2, HW-2004, VL-738, HS-240, MACS-2846, K-8027, Kharchia-65, KRL-99, KRL-210, HI-1544, HD-2380, EC-556429, E-1985, HI-8498
29-32 kDa	32, 31, 30, 29	39	VL-421, PBW-396, HUW-206, HS-295, RAJ-3765, A-9-30-1, KRL-19, WL-711, HDR-77, GW-496, PDW-233, RAJ-1555, Kalyan Sona, HI-1500, GW-173, PBW-533, DL-788-2, K-9107, Lerma Rojo, NIAW-34, DBW-17, PBW-34, NP-200, WH-291, PBW-226, HI-977, PDW-215, UP-2338, Sonalika, HW-2004 , HD-2329, VL-738, HI-8498, KRL-99, Kharchia-65, KRL-210, WH-1021, EC-556429, E-1985
	32, 30, 29	12	GW-322, HUW-234, PBW-343, MACS-6145, DDK-1009, PBW-373, PBW-175, GW-190, Kundan-DL-153-2, MACS-2846, HD-2851, EC-559464
	32, 31	20	PBW-502, K-9465, UP-262, GW-273, WH-542, HD-2285, HD-2733, HI-8381, WH-147, DBW-16, PBW-291, Chhoti Lerma, HD-2687, DWR-162, K-7903, HD-2833, NW-1014, PBW-299, UP-2425, EC-556441
	32, 31, 30	15	HD-4672, HD-2189, DWR-195, RAJ-3077, LOK-1, C-306, WH-896, WH-533, NW-2036, HS-240, VL-616, K-8027, HI-1544, HD-2380, EC-519484

seed protein fractions- albumins and globulins, gliadins, glutenins (extracted in NaOH), glutenins (extracted in SDS) and run on SDS-PAGE for assessment of polymorphism level in seven wheat lines. Significant differences were found based on electrophoretic phenotypes and zymograms.SDS-PAGE electrophoresis is a very useful technique for effectively describing the genetic structure of crop germplasm (Ciaffi *et al*. 1993). According to Levy *et al*. (1988), variations in the glutenin subunit of wild species can be used in plant breeding. Gliadin to glutenin ratio is the most important contributing factor for the rheological property of dough (Janssen *et al*. 1996).

Table 2: The variation in the polypeptide patterns of glutenin fraction of eighty-six Indian wheat genotypes on SDS-PAGE.

Molecular weight region	Molecular weights of polypeptides (kDa)	No. of lines	Wheat lines
75-110 kDa	110, 95, 80, 75	36	VL-421, HUW-206, RAJ-3765, WL-711, HDR-77, GW-496, GW-322, PBW-343, K-9465, DDK-1009, PBW-373, PBW-175, GW-190, PBW-533, DL-788-2, UP-262, GW-273, HD-2285, WH-147, DBW-16, HD-2687, RAJ-3077, LOK-1, K-7903, HD-2833, NW-1014, PBW-226, Kundan-DL-153-2, K-8027, HD-2851, HI-1544, HD-2380, WH-1021, EC-556441, EC-559464, E-1985
	110, 95, 75	24	PBW-396, KRL-19, HD-4672, PBW-502, DWR-195, Chhoti Lerma, DWR-162, WH-291, C-306, WH-533, PBW-299, HD-2733,UP-2425, NW-2036, Sonalika, HW-2004 , HD-2329, VL-738, HS-240, VL-616, Kharchia-65, KRL-99, KRL-210, EC-556429
	110,80,75	5	HS-295, MACS-6145, WH-542, HI-977, UP-2338
	95, 80, 75	9	HUW-234, HD-2189, KalyanSona, HI-1500, GW-173, K-9107, Lerma Rojo, NIAW-34, DBW-17
	110, 95	2	NP-200, A-9-30-1
	80, 75	5	PDW-233, RAJ-1555, PBW-34, PDW-215, MACS-2846
	95	3	HI-8381, PBW-291, HI-8498
	95, 75	2	EC-519484, WH-896
40-66 kDa	66, 60, 45, 40	40	VL-421, PBW-396, HUW-206, HDR-77, GW-496, GW-322, PBW-343, K-9465, HD-2189, PBW-373, PBW-175, GW-190, KalyanSona, DBW-17, GW-273, DWR-162, RAJ-3077, LOK-1, C-306, K-7903, HD-2833, NW-1014, PBW-299, PBW-226, HI-977, UP-2338, NW-2036, Kundan-DL-153-2, HD-2329, VL-738, HS-240, HI-8498, VL-616, K-8027, HD-2851, HI-1544, HD-2380, WH-1021, EC-556459, EC-556441
	60, 45, 40	23	HS-295, A-9-30-1, HD-4672, MACS-6145, DDK-1009, PDW-233, RAJ-1555, HI-1500, GW-173, DL 788-2, K-9107, PBW-34, NP-200, UP-262, DWR-195, HD-2733, HI-8381, DBW-16, WH-291, WH-533, PDW-215, MACS-2846, EC 519484
	66, 60, 40	13	RAJ-3765, KRL-19, WL-711, HUW-234, PBW-502, PBW-533, HD-2285, WH-147, HD-2687, Kharchia-65, KRL-99, KRL-210, E-1985
	60, 50, 45, 40	4	Lerma Rojo, WH-545, PBW-291, WH-896
	66, 60, 50, 45, 40	6	NIAW-34, Chhoti Lerma, UP-2455, Sonalika, HW-2004, EC 559464
22-36kDa	36, 34, 32, 30, 22	70	VL-421, PBW-396, HUW-206, HS-295, HDR-77, GW-496, HUW-234, PBW-343, PBW-502, K-9465, MACS-6145, DDK-1009, HD-2189, PBW-373, PDW-233, PBW-175, GW-190, RAJ-1555, HI-1500, GW-173, PBW-533, DL-788-2, K-9107, Lerma Rojo, NIAW-34, DBW-17, PBW-34, NP-200, UP-262, DWR-195, GW-273, WH-542, HD-2285, HD-2733, WH-147, DBW-16, PBW-291, Chhoti Lerma, HD-2687, DWR-162, RAJ-3077, WH-291, LOK-1, C-306, WH-896, WH-533, K-7903, HD-2833, NW-1014, PBW-299, UP-2425, PBW-226, HI-977, PDW-215, UP-2338, NW-2036, Sonalika, Kundan-DL-153-2, HW-2004, HD-2329, VL-738, HS-240, VL-616, K-8027, HD-2851, HI-1544, WH-1021, EC-556429, EC-556441, E-1985
	34, 32, 30, 22	6	RAJ-3765, A-9-30-1, WL-711, GW-322, HD-4672, KalyanSona
	36, 32, 30, 22	10	KRL-19, HI-8381, HI-8498, MACS-2846, Kharchia-65, KRL-99, KRL-210, HD-2380, EC-519484, EC-559464

CONCLUSION

It has been concluded from our study that based on the protein profiling of gliadin and glutenins (HMW-Gs and LMW-Gs), which plays an important role in dough quality, could be used as a better method for characterizing wheat lines. The gluten strength and extensibility are two main basic properties for the development of new cultivars. In our study, we clustered wheat cultivars according to gliadin and

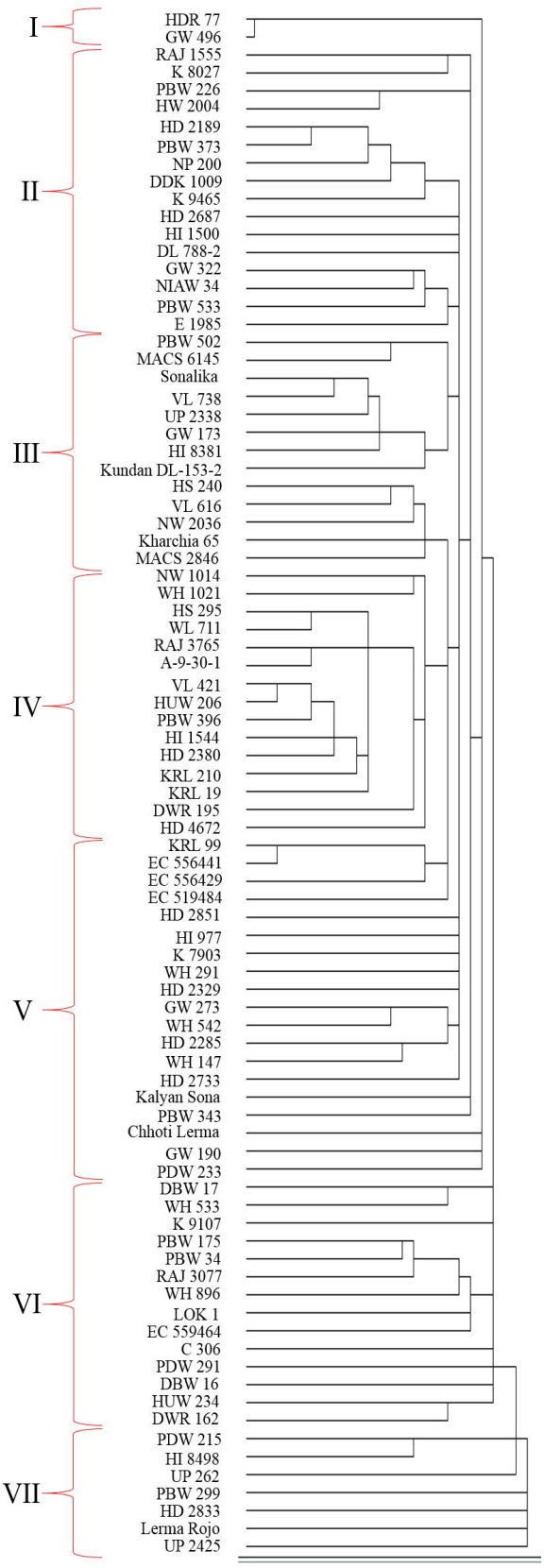


Fig 3: Genetic relationship of eighty-six wheat genotypes based on gliadin fraction.

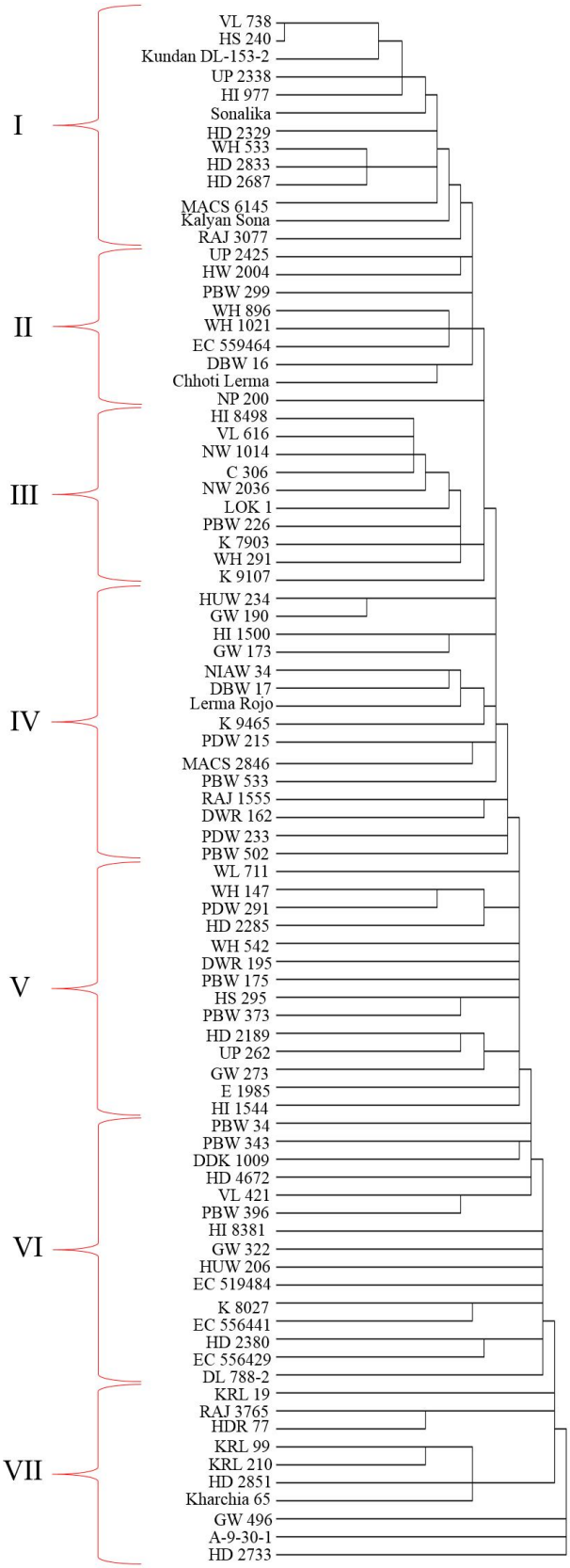


Fig 4: Genetic relationship of eighty-six wheat varieties based on glutenin fraction.

glutenin protein profiling in the dendrogram. From this dendrogram, we can compare the protein profiling and easily develop the wheat lines with improved bread-making quality and can also screen the wheat genotypes for the better bread-making quality.

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