Characterization of Hexaploid Wheat Protein on the Basis of their Date of Release in Indian sub-continent

Project submitted

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In

Life science (Biochemistry)

By

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DECLARATION

I declare that the project report entitled "**Characterization of Hexaploid Wheat Protein on the Basis of their Date of Release in Indian sub-continent**" has been prepared by me under the guidance of Dr. Monisha Dhiman, Associate Professor, Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this report has formed the basis for the award of any degree or fellowship previously**.**

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CERTIFICATE

I certify that Arti Negi has prepared her project report entitled **"Characterization of Hexaploid Wheat Protein on the Basis of their Date of Release in Indian sub-continent"** for the award of MSc. degree from the Central University of Punjab, under my guidance. She has carried out this work at the Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda.

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ACKNOWLEDGEMENT

Although only one name appeared on the cover of this M.Sc. Project but there are many hidden names that helped me to make contents in between the covers. I take this opportunity to express my thankfulness to all of them.

Although I did not find words that could express my thanks to a comparable extent for the help and support that she provided me but still trying to thank my guide Dr. Monisha Dhiman, who guided my M.Sc. and provided all facility to complete my Project. I have no words to express my gratitude for her. She has motivated me very much and this M.Sc. work would not have been completed without her help. I thank you, Maam, for all the help and advice (both personal as well as professional) you have given me at right time and in the right direction.

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ABSTRACT

Characterization of Hexaploid Wheat Protein on the Basis of their Date of Release in Indian sub-continent

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Key words: Gluten, Prolamins, Gliadin, Glutenin, Celiac disease, Cluster analysis

Wheat is the third most grown cereal worldwide. The storage proteins of wheat represent an important source of food and energy and are also involved in the determination of bread wheat quality. These gluten proteins are categorized as prolamins, composed of monomeric gliadin (single chain polypeptides) and polymeric glutenin (multiple polypeptide chains).Unfortunately consumption of these gluten protein is known to be linked with range of clinical disorders e.g. celiac disease, wheat allergy and wheat intolerance. The main objective of the present work is to elaborate a detailed knowledge of the variability of proteins and protein fractions of the wheat varieties based on their origin. 25 wheat varieties procured from Indian Institute of Wheat and Barley Research were evaluated for analysis of total wheat protein and gluten protein by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) and cluster analysis was done based on SDS-PAGE gels to investigate variation among varieties. Multiple sequence alignment of α-gliadin protein of different genes was done and region of highest variability among them is reported. This study shows that 25 varieties of wheat of different origin vary in their total wheat protein as well as gluten content. However the results of cluster analysis of gluten showed low degree of heterogeneity among the varieties.

(Arti Negi) **(Dr. Monisha Dhiman)**

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LIST OF ABBREVIATIONS

S.NO	Full form	Abbreviation
1.	Low molecular weight glutenin	LMW
2.	High molecular weight glutenin	HMW
3.	Immunoglobulin E	IgE
4.	Wheat-dependent exercise-induced anaphylaxis	WDEIA
5.	Human leukocyte antigen	HLA
6.	Celiac disease	CD
7.	Non-celiac gluten sensitivity	NCGS
8.	Tissue transglutaminase	tTG
9.	Sodium dodecyl sulfate polyacrylamide gel electrophoresis	SDS-PAGE
10.	Dithiothreitol	DTT
11.	Ammonium persulfate	APS
12.	Tetremethylethylenediamine	TEMED
13.	Acid- polyacrylamide gel electrophoresis	A-PAGE
14.	Unweighted pair group method with arithmetic means	UPGMA

CHAPTER - 1

INTRODUCTION

1. Introduction

Wheat is one of the major cereals grown worldwide. Its high nutritional characteristics, technological properties and long shelf life make wheat staple food of millions of people. Wheat endosperm contains two gluten proteins- gliadin (monomeric) and glutenin (polymeric), which primarily function as storage proteins. Gluten proteins are an example of a constituent whose suitable and usable characteristics are combined with properties that are sometimes harmful to human health. These proteins are classified as “prolamins” because of presence of high content of proline and glutamine in them. The molecular weight range of gliadin and glutenin protein is 35-74kDa and 30-140kDa(Žilić *et al.*, 2011).Gliadin is categorized into different groups α, β, ɣ and Ω based on their molecular weight and electrophoretic mobility at acidic pH. Glutenin can be subdivided into low molecular weight glutenin (LMW) and high molecular weight (HMW) glutenin. High molecular weight glutenin (HMW) comprises of two subunits- “x” of high molecular weight and “y” of low molecular weight(Payne *et al.*, 1981). Although gliadin and glutenin are two different fractions of gluten proteins but they share structural similarities (i.e contain specific amino acid sequences).

Food products made of wheat seed are common in human diets, thus gliadin and glutenin are of great nutritional importance but in the recent years consumption of gluten proteins has been linked to various clinical disorders. Wheat allergy, celiac disease and wheat intolerance exhibit different gluten-related disorders. Celiac disease is an enteropathy triggered by ingestion of gluten proteins. α- and ɣ-gliadin seems to the major initiators of celiac disease and unfavorable effects of these two gliadin in humans occur only in genetically sensitized individuals. Wheat allergy is defined as an IgE- mediated immune reaction to wheat proteins. Unlike wheat allergy wheat intolerance does not involve immune system and symptoms tend to occur after a longer period of time.

Presence of certain amino acid sequences rich in proline and glutamine residues are significant elements of primary structure of prolamins. These residues are responsible for their allergenic properties.(Waga, 2004) have reported that allergies are associated

with pentapeptide Gln-Gln-Gln-Pro-Pro, and celiac disease with tetra peptides Gln-Gln-Gln-Pro and Pro-Ser-Gln-Gln.

The main objective of the present work is to study in detail about the variability of proteins and protein fractions of the 25 wheat varieties based on their origin which can facilitate and improve our knowledge about the antigenic properties of gluten and its associated proteins that can be associated with wheat protein intolerance disease like celiac disease (CD) or non-celiac gluten sensitivity (NCGS).

1.1 KNOWLEDGE GAP

The selection for resistance to diseases and pests, high yield in crop plants, could have implied the selection of particular alleles of prolamins, as genes for disease resistance are distributed in gene rich regions all over the wheat genome, including those in group 1 and group 6 chromosomes where gliadin loci are encoded. During the process of domestication the possibility of increased number of toxic peptides is there. Till date no study has been done to screen the Indian wheat varieties based on origin to screen their proteins and metabolites.

1.2 HYPOTHESIS

The domestication of wheat is the result of a previous natural interspecific hybridization first between diploid, and then between diploid and tetraploid species that resulted in hexaploid wheat. In the modern day wheat the traits selected are for the better adaptation and high yield but during breeding the gliadin-related genes, responsible for triggering wheat protein associated intolerance may also have been increased unconsciously. We hypothesize that variation of protein expression from various wheat varieties of hexaploid origin might be due to repetitive breeding which may have a significant role in gluten related disorders.

1.3 OBJECTIVES

1. To screen the various wheat varieties on the basis of their origin for the protein profile and to examine the sequence similarities and degree of conservation at amino acid level for gliadin protein.

2. To analyze the correlation among various wheat varieties on the basis of their protein profile and secondary metabolites.

CHAPTER - 2

REVIEW OF LITERATURE

2. Review of Literature

Wheat is a crop of global significance and is the third most grown cereal worldwide after rice and maize. Its high nutritional characteristics, technological properties and long shelf life make wheat staple food of millions of people. Wheat is a polyploidy complex formed by multiple species of different ploidy level, consequence of breeding between different species of the *Triticeae* tribe. Around 8,000 years ago bread wheat or hexaploid *Triticum aestivum* (AABBDD) originated from a hybridization of a tetraploid *Triticum* species with the diploid donor of the D genome *T.tauschii.* The A and B genomes were most likely contributed by *T.turgidum;* itself possibly resulted from hybridization between wild diploid *T.monococcum* (A genome) and the donor of the B genome (van Herpen *et al.*, 2006).

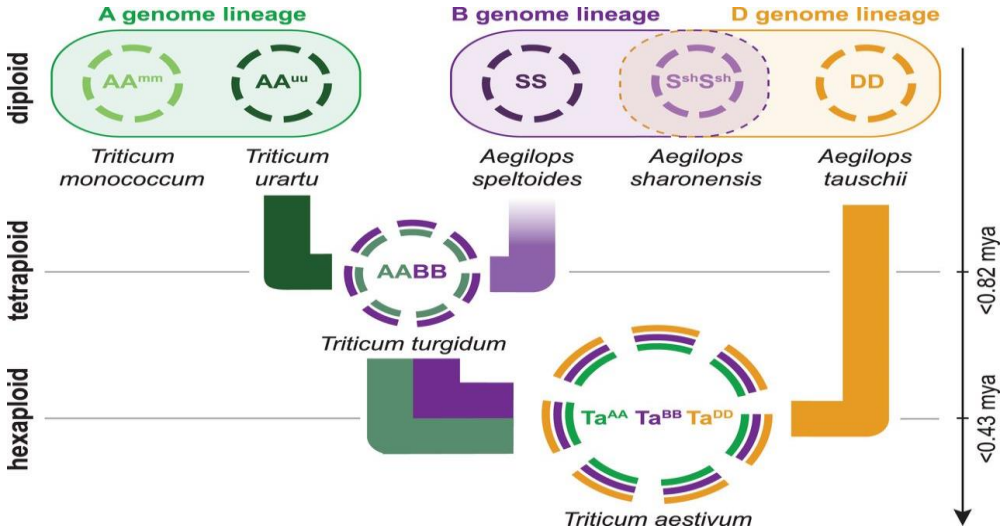

Fig 2.1. Schematic diagram of the relationships between wheat genomes with polyploidization history and genealogy. Source(International Wheat Genome Sequencing Consortium, 2014).

A wheat kernel comprises three principle fractions – bran, germ and endosperm.

- Bran – It is the outer most layer of the grain containing fiber, antioxidants, B vitamins, and 50-80% of minerals in grains.
- Endosperm- It is the middle layer of the grain containing mostly carbohydrates, protein, and small amounts of some B vitamins and minerals.
- Germ- It is the inner most component of the grain rich in healthy fats, B vitamins, phytochemicals, and antioxidants like vitamin E. [http://www.health.state.mn.us/divs/hpcd/chp/cdrr/nutrition/facts/wholegrains.html]

Wheat seed endosperm contains mainly four protein fractions soluble in different solvent. They are:

1) Albumins, soluble in water.

2) Globulins, soluble in dilute salt solutions.

Gluten Proteins 3) Gliadins, soluble in 70% ethanol

4) Glutenins, soluble in acid solutions

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2.1 GLUTEN

Gluten can be defined as the rubber like mass which is remained when dough is washed to remove starch granules and water-soluble constituents. It contains hundreds of protein components present either as monomers or as oligomers and polymers. The molecular weights (MWs) of proteins range from around 30,000 to more than 10 trillion(Bietzand Wall, 1980).

Gluten proteins classified as prolamins are composed of monomeric gliadin and polymeric glutenins. They are called prolamins because of presence of their high content of proline and glutamine. Prolamins function primarily as storage proteins.

1) Gliadin (prolamins I) - comprises of 40-50% of total endosperm storage proteins in wheat; monomeric polypeptides with weak H- bonds
2) Glutenin (prolamins II) - polymeric complexes formed by covalent intermolecular disulphide bonds.

2.2 Gliadins and Glutenins

Gliadins form a large family of proteins. Although all of the gliadins share similar chemical and physical attributes but they are extremely heterogeneous with respect to their molecular weight and net charge. Gliadins are classified into four groups α, β, ɣ and Ω based on their molecular weight and electrophoretic mobility at acidic pH. The molecular weight of ω-gliadins is in the range of 46 and 74 kDa and the α-, β- and γ- gliadins have lower molecular weight ranging from 35 to 45 kDa.(Žilić *et al.*, 2011). Each gliadin type is coded by genes present on specific chromosomes- heaviest ω- gliadins are coded by genes present on chromosome 1D; lighter ω-gliadins by chromosomes 1A, 1B, 1D and 6B; β gliadins by chromosomes 6B and 6D; and γ gliadins by chromosomes 6A and 6D (Waga, 2004).Members of a multigene family are known to encode α-gliadin proteins. α/β-type gliadin subfamilies are localized on the short arm of 6A, 6B and 6D wheat chromosomes(Shewry *et al.*, 1992).

Glutenins, another gluten protein, can be subdivided into low-molecular weight glutenin subunits (LMW-GS) and high-molecular weight glutenin subunits (HMW-GS) with molecular weight range of 100 to 140 kDa and 30 to 55 kDa (Žilić *et al.*, 2011). HMW glutenin contains two subunits: x – with high molecular weight and, y- with low molecular weight (Payne *et al.*, 1981).

Gluten proteins can also be classified as Sulphur (+) and Sulphur (-) due to presence of cysteine residues in their sequences. α, β and γ gliadins contain cysteine residues and thus are defined as protein rich in Sulphur.

Heaviest gliadins protein-ω-gliadins contain no cysteine residues and so are defined as protein deficient in Sulphur.

2.3 Regions and domains of gluten proteins

Gliadin and glutenin proteins share structural similarities in that they both contain specific repeatable amino acid sequences, formed from short peptides. The only difference in their structure is in the arrangement, size and sequence of the repeated regions. Alpha gliadin proteins contain repeatable sequence of 5 amino acids (Pro-Gln-Gln-Pro-Phe-Pro) which is preceded by a very short N-terminal region and followed by C-terminal region. Primary structure of α-gliadins also contain two domains rich in glutamine. These two domains have specific locations. One of these domains is present at the border of the specific repeatable sequences, and the other is present in the middle of the specific sequences region. Structure similar to α-gliadin has been found for β and γ gliadins. In β- gliadin the sequence of repeatable regions is formed of Pro-Gln-Gln-Pro-Tyr and in γ- gliadin the sequence of repeated regions is Pro-Gln-Gln-Pro-Phe-Pro-Gln. ω- gliadin protein contain N terminal sequence followed by repeatable sequence of 8 amino acids and short, distinct C terminal sequence. Sequences of repeated regions in ω-gliadin are similar to γ-gliadins with an additional Gln amino acid at the end of the peptide in omega gliadins. HMW subunits comprise of 3 domains- a central repetitive domain flanked by non- repetitive N- and C-terminal domains. Central repetitive domain comprises tripeptide, hexapeptide and nonapeptide motifs (in x- type only). The differences in the size of HMW subunits result mainly from the differences in the number of hexapeptides and tripeptides, present in the repetitive domain (Shewry *et al.*, 1992) .

Presence of amino acid sequences rich in proline and glutamine residues are significant elements of primary structure of prolamins and are related to their allergenic

properties. Allergies are associated with pentapeptide Gln-Gln-Gln-Pro-Pro, and celiac disease with tetra peptides Gln-Gln-Gln-Pro and Pro-Ser-Gln-Gln (Waga, 2004).The pentapeptide Gln-Gln-Gln-Pro-Pro, associated with allergies, has specificity for binding to IgE antibodies in serum of patients with allergic symptoms (Waga, 2004). Gliadins represent important allergens and different classes of gliadins are involved in IgE binding. (Matsuo *et al.*, 2004) identified four dominant epitopes QQIPQQQ, QQFPQQQ, QQSPEQQ and QQSPQQQ on ω5-gliadin for patients with WDEIA, with critical amino acids at position 1 and 4 to 7. The high content of Gln and Pro residues in gluten proteins makes them resistant to proteolytic digestion by gastric, pancreatic and intestinal enzymes. Presence of multiple Gln and Pro residues in gluten proteins are characteristics of their celiac disease (CD) triggering sequences and also make them preferred substrate for the enzyme tissue transglutaminase (tTG). tTG specifically deamidates certain Gln residues of gluten, increasing their affinity for HLA-DQ-2 or HLA-DQ8 molecules. This generates an enhanced CD4+ T-helper 1 T-cell activation, resulting in villous atrophy, nutrition malabsorption and numerous secondary symptoms. tTG acts as an autoantigen in celiac disease (Dieterich *et al.*, 1997)

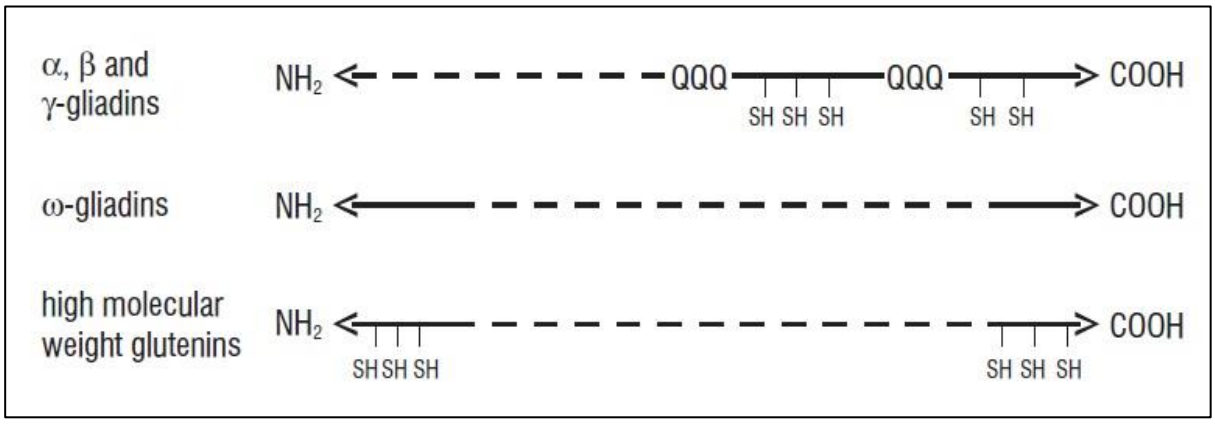

Fig 2.2 Schematic diagram of gliadin and glutenin polypeptides. Regions of specific (continuous line) and repeated (dashed line) sequences, polyglutamine regions (QQQ) and cysteine residues (SH) are marked. Source (Waga, 2004)

2.4 Wheat protein related disorders

Food items made of wheat are common in human diet. In the recent years wheat ingestion has been associated with a range of clinical disorders. Intake of wheat is associated with both intolerances and allergies. Wheat allergy, celiac disease and wheat intolerance exhibit different gluten-related disorders. Celiac disease is an enteropathy triggered by ingestion of gluten. In this gluten derived peptides evoke a T-cell mediated autoimmune reaction in genetically susceptible individuals (HLA-DQ2 or HLA-DQ8 positive). HLA-DQ2 and HLA-DQ8 belongs to class II HLA molecules and bind peptides of variable length, usually 12-20 amino acids long (Elli *et al.*, 2015). α-gliadins appear to be the major initiators of celiac disease (CD), affecting as many as one in every 300 people (Shewry *et al.*, 1992). Wheat allergy is defined as an IgE-mediated immune reaction to wheat proteins. Based on route of allergen exposure, wheat allergy can be classified into occupational asthma or baker's asthma, wheat dependent exercise-induced anaphylaxis (WDEIA) and contact urticarial. Unlike wheat allergy, wheat intolerance does not involve the immune system. The exact mechanism of wheat intolerance is still unclear.

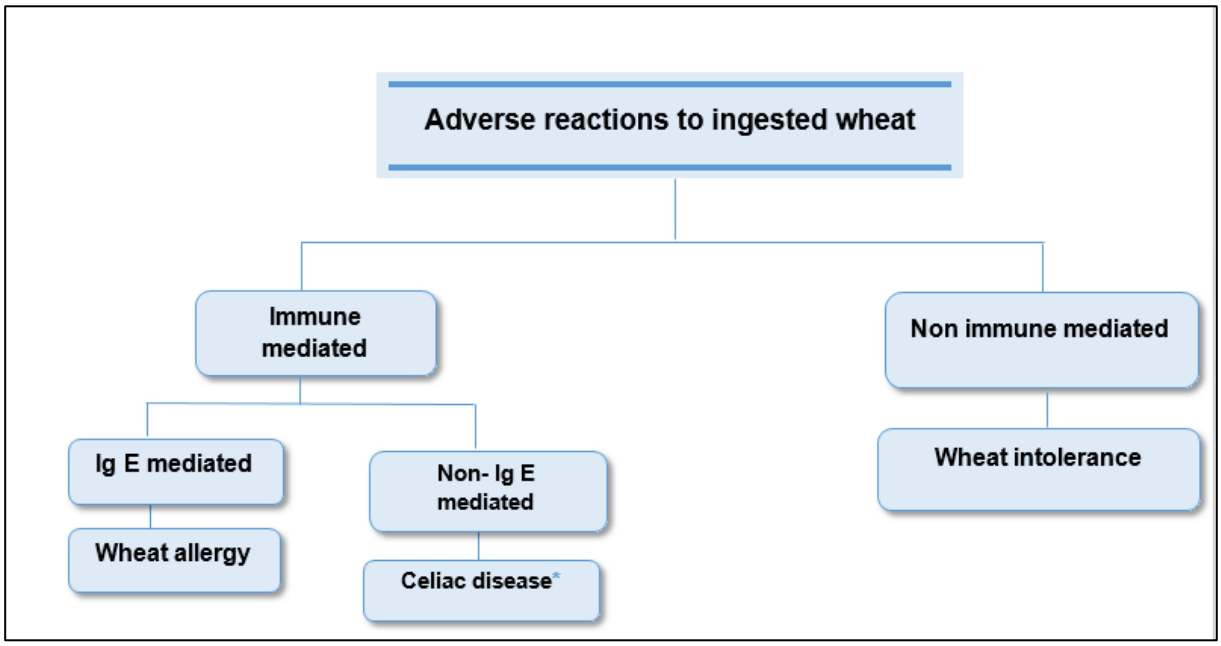

Fig 2.3 Flowchart showing various adverse reactions of ingested wheat

*The exact classification of coeliac disease and whether it should be classified as a non-IgE mediated allergy or a food intolerance much debated in allergy and gastro-enterology communities.

2.5 Prevalence of Wheat protein disorders

Celiac disease is widespread across globe with an incidence rate of 1% worldwide. The occurrence of CD is more in western countries. It is around 1% of the general population in many western countries. The incidence rate of CD is highest in Sahara people of Africa because of their highly susceptible genotypes. CD is virtually not found in East Asian population. In India, CD is more prevalent in North Indian population where wheat is primarily grown and is consumed by majority of the population. Until now CD has no prevalence in South Indian population but in recent reports CD cases have been diagnosed in South India. [http://celiacindia.org.in/about-celiac-disease/celiac-disease/prevalence/].

2.6 Secondary Metabolites

Secondary metabolites are structurally different and many are distributed among a little number of species within a plant kingdom and so can used as a diagnostic tool in chemotaxonomic studies. The knowledge of lipid components of wheat finds an important information in order to differentiate between different wheat varieties (Armanino *et al.*, 2002) .

CHAPTER - 3

MATERIALS AND METHODS

3.1. Wheat Germplasm used in this study

Wheat (*Triticum aestivum)* varieties of hexaploid origin used in this study were procured from Indian Institute of Wheat and Barley Research, Karnal. The year of release/ cultivation of these wheat varieties are from year 1965 – 2010.

S.No	Variety Name	Year of release	S.No	Variety Name	Year of release
1.	C306	1965	16.	HUW 206	1983
2.	Chottilerma	1967	17.	LOK1	1981
3.	Kalyansona	1967	18.	VL 616	1986
4.	Lermarajo	1965	19.	HS 295	1992
5.	Sonalika	1965	20.	PBW 343	1995
6.	HD 2009	1975	21.	RAJ 3765	1995
7.	HD 2189	1979	22.	UP 2338	1994
8.	UP 262	1977	23.	GW 322	2002
9.	VL 421	1979	24.	PBW 502	2003
10.	WH 157	1978	25.	DBW 39	2010
11.	WL 711	1977			
12.	HD 2285	1983			
13.	HD 2329	1982			
14.	HI 617	1983			
15.	HS 240	1989			

Table 3.1. List of wheat varieties used in the present work

3.2. Extraction of total wheat proteins

Procedure: 25 wheat samples, each weighing 5 grams, were ground into a powder using a grinder.100mg of grinded wheat was mixed with 1.5ml SDS buffer (2%SDS, 50 mM DTT, 10%glycerol, and 40mMTris-Cl pH6.8) and was incubated for 1 hr at RT with intermittent mixing. After incubation samples were centrifuged at 16000g for 10min at 4^0C. Total wheat proteins were obtained as supernatant.

3.3. Separation of gluten protein from wheat flour

Flour proteins are separated into the gluten fraction based on solubility in KCl buffer and SDS buffer.

Procedure: 250mg grinded wheat grain was dissolved in 200µl cold KCl buffer and centrifuged for 15 min at 4^0C.After centrifugation pellet was treated with SDS buffer (2%SDS,10% glycerol,50mM DTT, 40mM Tris (pH 6.8) and centrifuged again. The pellet so obtained this time was discarded and supernatant was treated with cold acetone and incubated overnight at -20*C. The mixture was centrifuged again for 10min. Finally, the pellet obtained was dissolved in the urea buffer, giving the gluten protein.

3.4. Isolation of Gliadin from wheat flour

Protein fraction of gluten i.e gliadin is separated on the basis of its solubility in ethanol which is soluble in 70% ethanol.

Procedure: Grinded wheat seed was treated with 70% ethanol and incubated at RT for 30 min. The homogenate was then centrifuged at 8000g for 5 min at RT. The supernatant obtained is the Gliadin proteins which was stored as aliquots till further use.

3.5. Bradford Assay for Protein Estimation

Bradford assay is done to quantify protein concentration present in a given sample. It is a simple, fast and sensitive assay. The principle underlying Bradford assay is the binding of the Coomassie blue G-250 dye to proteins.

Procedure: The standard curve was prepared using serial dilution (0.01-0.2 mg/ml) of BSA (stock of BSA 1mg/ml). The protein sample (5µl) was added to 75 µl of (1X) PBS to make final volume 80 µl and to this 20 µl of Bradford reagent was added to make final volume 100 µl. After incubation of 10-15min, the blue color formed was measured at 595nm wavelength with Synergy H1 micro plate reader.

3.6. SDS – PAGE for characterization of total wheat protein and gluten protein

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis is an analytical method used to separate components of a protein mixture based on their molecular weights. The basic principle of SDS-PAGE is SDS, an anionic detergent, masks the overall charge present on the surface of the protein and therefore under electric field proteins are separated based on their molecular weight. A solution of acrylamide and bis-acrylamide is polymerized to form gel. Acrylamide forms linear polymers and bis-acrylamide introduces crosslinks between polyacrylamide chains. The pore size of the gel is determined by the ratio of acrylamide to bis-acrylamide, and by the concentration of acrylamide. Polymerization of acrylamide and bis-acrylamide monomers is induced by ammonium persulfate (APS). TEMED is a free radical stabilizer and is used to promote polymerization (Gupta *et al*., 2018).

Procedure: 25µg each of total wheat protein samples and 50 µg each of gluten protein samples were separated by polyacrylamide gel electrophoresis under denaturing conditions. The standard molecular weight marker of 250-10kDa was used. Electrophoresis was performed at 70volts at RT. The gels were stained overnight with 0.2% Coomassie blue G-250 staining solution. The gels were destained in destaining solution and images of gel were taken on advanced gel documentation system (BioRad).

3.7. Acid – PAGE

Acid-polyacrylamide gel electrophoresis is a useful method to separate different components of gliadin. Electrophoresis at acid pH (A-PAGE) has been used to analyze and characterize gliadins. A-PAGE separates protein on the basis of both molecular weight and charge. Therefore two proteins of similar size but different charge may be separated by this technique. Urea increases the frictional coefficient of proteins and so alters their electrophoretic mobilities (De Villiers *et al*.,) (De Villiers & E.W,1988).

Procedure: 20g urea was dissolved at 40^{o} C, gently shaken, and 1M sodium acetate (pH 5.0) and 40% acrylamide, APS (20%) was added to make up the volume to 40ml. To the above solution TEMED (23µl) was added and this was then poured into the gel cassettes setup [D.T. de Villiers* and E.W. Laubscher]. Electrophoresis was done at 70V at RT. Gels were stained overnight with 0.2% Coomassie blue G-250 to visualize the protein bands. Images were taken on advanced gel documentation system (BioRad).

3.8. Densitometry

Protein gel bands obtained after electrophoresis were analyzed and compared using Image lab5.2.1 software. Gluten bands of C306 (one of the oldest variety used in the present work) were compared with the rest 24 varieties. Presence of additional bands/ absence of bands and fold change of bands with same molecular weight was calculated and reported.

3.9. Gas chromatography- Mass spectrometry (GC-MS)

Gas chromatography coupled to mass spectrometry is an analytical tool to separate, quantify and identify unknown (volatile) organic compounds. The GC works on the principle that upon heating mixture get separated into individual substances. The heated gases are carried through a column with an inert gas. As the separated substances come out of the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule.

Principle: Sample preparation- 100mg each of 25 samples of finely grinded wheat grain was mixed with 5ml n- hexane and vortexed for 2-5 min. After vortexing, it was centrifuged at 2000rpm for 5 min and supernatant was taken. Supernatant was dried completely in the speed vac (30^0C, vacuum).1.2ml Dichloromethane and methanal in the ratio 9:1 + 20µl TMSH (trimethylsulfonium hydroxide) was added to dried supernatant and mixed and centrifuged at 10000 rpm for 5 min. 1ml of clear supernatant free of debris was taken and injected into the GC (Shimazdu) (Vujić *et al.,* 2012).

3.10. Multiple Sequence Alignment

The protein sequences of α gliadin protein of different genes of *Triticum aestivum* were collected from Uniprot database and compiled. Multiple sequence alignment of the compiled sequences was conducted using Clustal Omega tool at European Bioinformatics Institute (EBI) (https://www.ebi.ac.uk/Tools/msa/). Default parameters were used for the analysis (Anwar *et al.,* 2013).

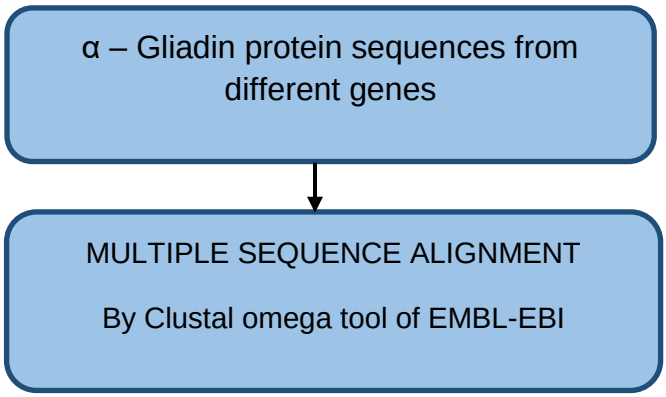

Fig 3.1 Overview of method

CHAPTER - 4

RESULTS

Results:

4.1. Estimation of protein in the wheat samples by Bradford method

The total amount of protein (concentration) present in 25 samples each of total wheat protein, Gluten protein and Gliadin protein was calculated by Bradford method using BSA as a standard.

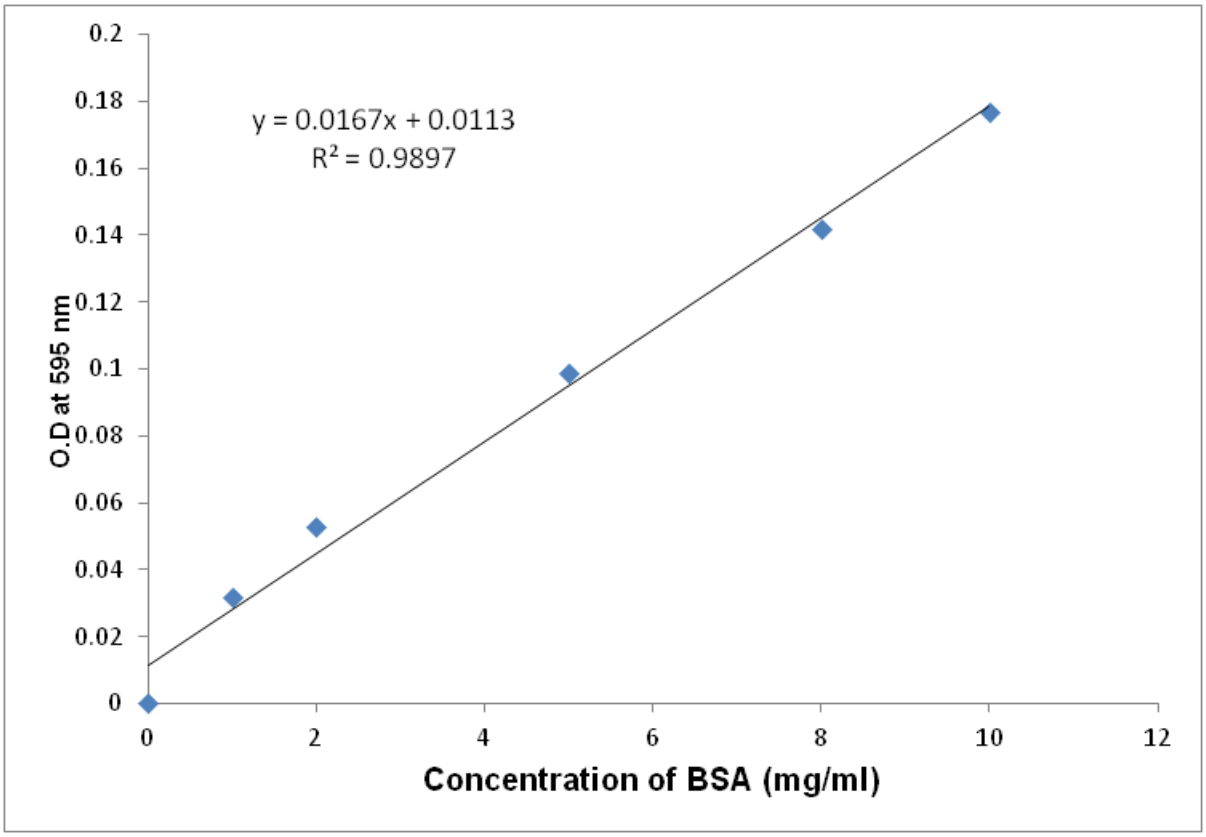

Figure 4.1: The standard curve of known concentration of BSA for the estimation of protein concentration by Bradford method.

The total wheat protein from 100 mg of wheat samples was in range of 0.45 to 1.86 µg/µl. The wheat varieties VL421, WH157 and WL 711 had the minimum protein of 0.82, 0.451 and 0.749 µg/µl respectively (**Table. 4.1a**). The amount of gluten in the wheat varieties was almost the same within the range of 3.62 to 4.84 µg/µl (**Table. 4.1b**). There was a variation in the total amount of gliadin in these 25 wheat varieties. Chottilerma, Lermarajo, VL421, HS 295 and VL616 varieties had very low amount of gliadin 0.070, 0.043, 0.065, 0.001 and 0.086 µg/µl whereas few varieties had a high gliadin concentration ranging from 0.102 – 0.347 µg/µl.

S.NO	Variety Name	O.D	Concentration (µg/µl)
1	C306	0.26	1.60
2	Chottilerma	0.234	1.19
3	Kalyansona	0.234	1.19
4	Lerma Rajo	0.2423	1.32
5	Sonalika	0.255	1.52
6	HD 2009	0.2673	1.72
7	HD 2189	0.276	1.86
8	UP 262	0.233	1.17
9	VL 421	0.211	0.82
10	WH 157	0.1877	0.45
11	WL 711	0.2063	0.74
12	HD 2285	0.2507	1.45
13	HD 2329	0.2537	1.50
14	HI 617	0.2607	1.61
15	HS 240	0.2557	1.53
16	HUW 206	0.262	1.64
17	LOK1	0.2507	1.45
18	VL 616	0.2427	1.33
19	HS 295	0.2313	1.14
20	PBW 343	0.225	1.04
21	RAJ 3765	0.253	1.49
22	UP 2338	0.258	1.57
23	GW 322	0.248	1.41
24	PBW 502	0.2713	1.78
25	DBW 39	0.2653	1.69

Table 4.1.a Amount of protein (concentration in µg/µl) in total wheat protein samples.

S.NO	Variety name	O.D	Concentration (µg/µl)
1	C306	0.341	3.94
2	Chottilerma	0.388	4.50
3	Kalyansona	0.35767	4.14
4	Lerma Rajo	0.38133	4.42
5	Sonalika	0.37867	4.39
6	HD 2009	0.35167	4.07
7	HD 2189	0.34833	4.03
8	UP 262	0.344	3.98
9	VL 421	0.31433	3.62
10	WH 157	0.35433	4.10
11	WL 711	0.416	4.84
12	HD 2285	0.369	4.28
13	HD 2329	0.39533	4.59
14	HI 617	0.35633	4.13
15	HS 240	0.358	4.15
16	HUW 206	0.35067	4.06
17	LOK1	0.388	4.50
18	VL 616	0.36067	4.18
19	HS 295	0.38167	4.43
20	PBW 343	0.36233	4.20
21	RAJ 3765	0.372	4.31
22	UP 2338	0.366	4.24
23	GW 322	0.38167	4.43
24	PBW 502	0.342	3.95
25	DBW 39	0.359	4.16

Table 4.1.b Amount of protein (concentration in µg/µl) in Gluten samples

S.NO	Variety name	O.D	Concentration(µg/µl)
1	C306	0.149333	0.12
2	Chottilerma	0.145667	0.07
3	Kalyansona	0.150667	0.15
4	Lerma Rajo	0.144	0.04
5	Sonalika	0.155	0.21
6	HD 2009	0.154	0.20
7	HD 2189	0.157	0.25
8	UP 262	0.158333	0.27
9	VL 421	0.145333	0.06
10	WH 157	0.151667	0.16
11	WL 711	0.152667	0.18
12	HD 2285	0.154667	0.21
13	HD 2329	0.152333	0.17
14	HI 617	0.160333	0.30
15	HS 240	0.15	0.13
16	HUW 206	0.163	0.34
17	LOK1	0.153667	0.19
18	VL 616	0.146667	0.08
19	HS 295	0.141333	0.001
20	PBW 343	0.148333	0.11
21	RAJ 3765	0.147667	0.10
22	UP 2338	0.155333	0.22
23	GW 322	0.158333	0.27
24	PBW 502	0.153	0.18
25	DBW 39	0.154333	0.20

Table 4.1.c Total amount of protein (concentration in µg/µl) in Gliadin samples**.**

4.2 Detection of variation in banding pattern in different protein samples by SDS-PAGE

The total wheat protein content and gluten protein content in each of the 25 samples revealed considerable variation. The protein band pattern which was separated on a SDS-PAGE showed a very peculiar pattern for total wheat protein, Gluten protein from different wheat varieties (Figures 4.2.a and 4.2.b). The differences in protein content might be due to repetitive breeding. Repetitive breeding may have resulted in loss/addition of protein bands throughout these years (1965-2010).

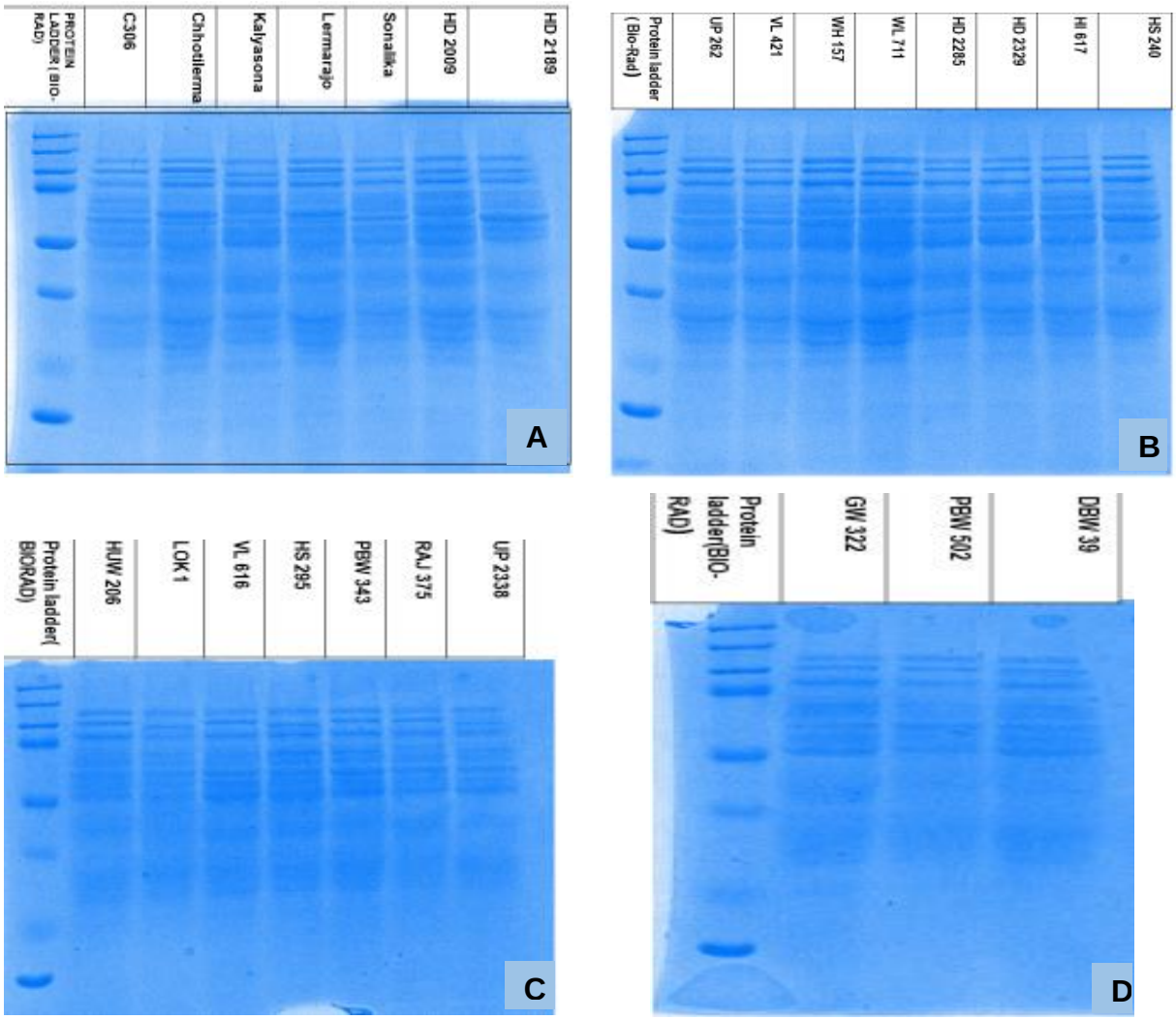

Fig 4.2.a.(A-D) Panel showing protein banding pattern of total wheat protein of different varieties.

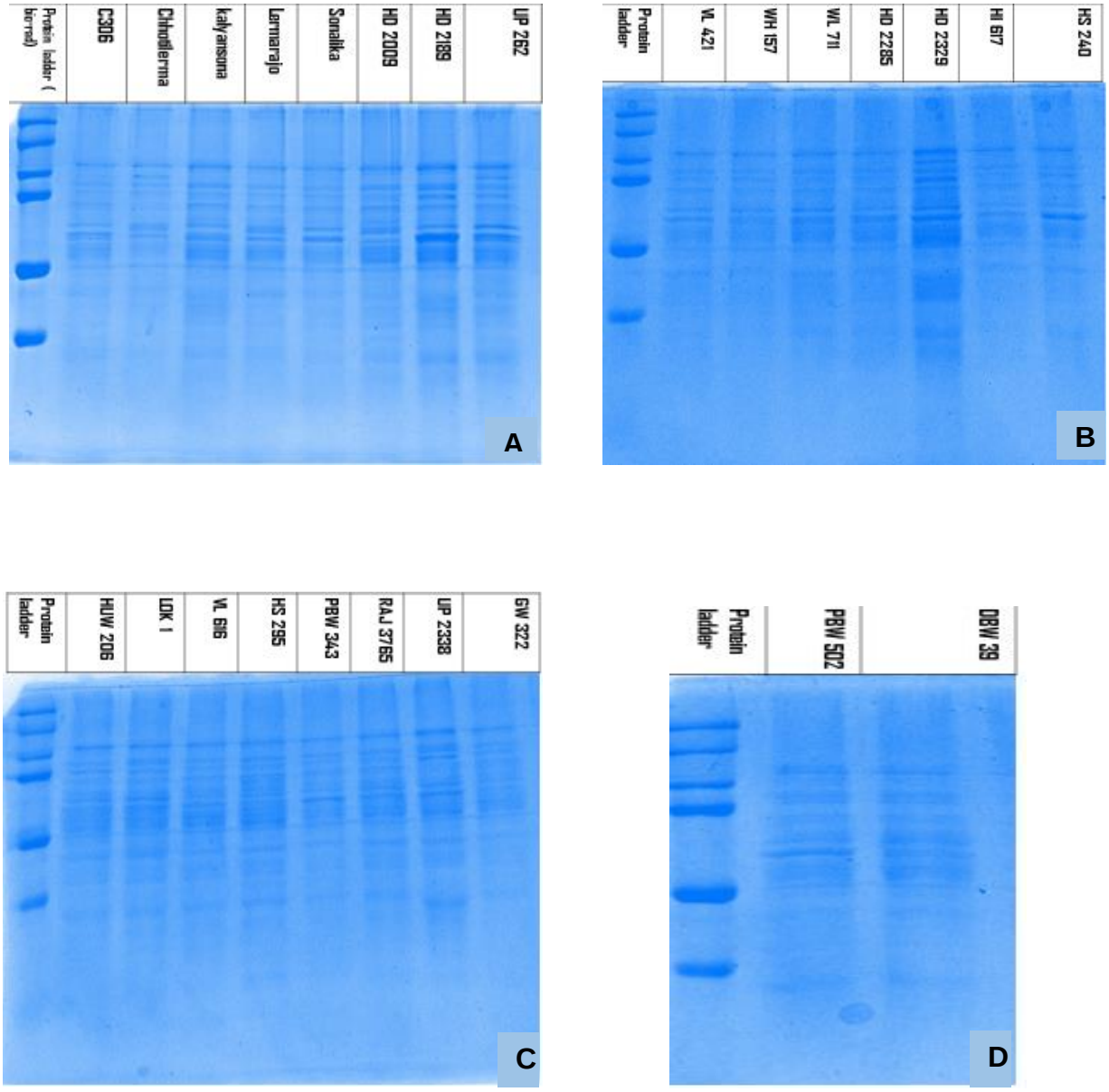

Fig 4.2.b (A-D) Panel showing protein banding pattern of Gluten protein of different varieties.

4.2.1. Analysis of bands of gluten samples by Densitometry

Bands of gluten protein samples obtained after SDS-PAGE were compared. The bands of the oldest variety (C306) when compared with the other 24 samples indicated considerable variation in the number of bands as well as in their intensities. In **Chhotilerma sample**, the band intensity of 6 bands were up-regulated in the range of 3-13 fold change whereas the intensity of 6 bands were down-regulated in from 2-19 fold (Table 4.2.a). In **Kalyansona sample** when compared with the oldest variety C306, band intensity of 9 bands were up-regulated and one of the up-regulated band of mol.wt 28kDa showed 22 fold up-regulation whereas the band intensity of 8 bands were down-regulated (Table 4.2.b).

In **Lermarajo** wheat variety, band intensity of 7 bands showed up-regulation by 2-9 folds and 7 bands were down-regulated when compared with the protein profile of oldest variety C306 (Table 4.2.c).

In **Sonalika sample** as compared to the oldest variety C306 band intensity of 9 bands were up-regulated by 2-11 fold whereas the intensity of 8 bands were down-regulated by 2-14 folds (Table 4.2.d).

In **HD2009 wheat variety** intensity of 10 bands showed up-regulation of 2-14 folds and 7 bands were down-regulated. One of the band in the mol.wt range 81-84 kDa showed 30 fold down-regulation (Table 4.2.e).

The **HD2189 wheat variety** when compared with the oldest variety C306, band intensity of 13 bands were up-regulated among which a band of mol.wt 28kDa showed maximum up-regulation of 40 fold whereas 7 bands were down-regulated (Table 4.2.f).

In **UP262 variety** when compared with the oldest variety C306, band intensity of 13 bands were up-regulated with one of the band of 28kDa showing maximum up-regulation of 27 fold .The band intensity of 3 bands showed down-regulation (Table 4.2.g).

The **VL421 wheat variety** when compared with the oldest variety C306, band intensity of 7 bands were showing up-regulation whereas and 13 bands were down-regulated.

Among the downregulated bands one of the band of 25kDa showed maximum downregulation of 85 fold (Table 4.2.h).

The **WH157 wheat variety** when compared with the oldest variety C306, band intensity of 3 bands were up-regulated and 15 bands were downregulated with one of the band of 25kDa showing maximum down-regulation of 45 fold (Table 4.2.i).

In **WL711 variety** the protein band intensity of 3 bands were up-regulated and 15 bands were down-regulated (Table 4.2.j). Among the down-regulated bands the maximum down-regulation of 44 folds was observed in one band of mol.wt 61kDa (approximately).

The **HD2285 wheat variety** when compared with the oldest variety C306, band intensity of 7 bands were showing up-regulation within a range of 2-11 fold change and 10 bands were showing down-regulation (Table 4.2.k).

In **HD2329 wheat sample**, 9 bands were up-regulated and 5 bands were down-regulated (Table 4.2.l).

The **HI617 wheat variety** when compared with the oldest variety C306, band intensity of 3 bands were up-regulated whereas the band intensity of 19 bands were down-regulated (Table 4.2.m).

In **HS240 sample** the band intensity of 6 bands were up-regulated and 7 were down-regulated when compared with the oldest variety C306 (Table 4.2.n). Among the down-regulated bands one band of mol.wt of 32kDa showed maximum down-regulation of 58 fold.

In **HUW206 wheat variety** the band intensity of 6 bands were up-regulated by 2-25.folds and only 1 band was down-regulated (Table 4.2.o).

The **LOK1 wheat variety** when compared with the oldest variety C306, band intensity of 3 bands were up-regulated and 1 band was down-regulated (Table 4.2.p).

In **VL616 sample** the band intensity of 4 bands were showing up-regulation and 2 bands showed down-regulation (Table 4.2.q).

In **HS295 sample** only one band was up-regulated and 4 protein bands showed down-regulation (Table 4.2.r).

The **PBW343 wheat variety** when compared with the oldest variety C306, the band intensity of 5 bands were up-regulated and only one band was showing down-regulation (Table 4.2.s).

In **RAJ3765 wheat variety** the band intensity of 6 bands were up-regulated and only one band was down-regulated (Table 4.2.t).

In **UP2338 wheat variety** the band intensity of 6 bands were up-regulated and 3 bands were down-regulated when compared with the oldest variety C306 (Table 4.2.u).

In **GW322 wheat variety** the band intensity of 6 bands were observed to be up-regulated and 4 bands were down-regulated (Table 4.2.v).

In **PBW502 sample** the band intensity of 5 bands were up-regulated by 3-25 folds and 5 bands were down-regulated (Table 4.2.w).

The **DBW39 wheat variety** when compared with the oldest variety C306, the band intensity of 3 bands were showing up-regulation whereas 5 bands were showing down-regulation with one of the band of mol.wt of 39kDa showed maximum downregulation of 34 fold (Table 4.2.x).

C306			Chhotilerma				
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/Down**
1	135.29	614241	1	127.54	625917	1.019	
2	111.69	218925	2	111.69	213921	1.023	
			3	101.48	42673		
3	94.40	9869	4	94.40	36557	3.70	↑
			5	90.03	19599		
4	81.07	646489	6	79.98	610627		
5	77.84	86180					
6	71.47	104528	7	69.53	308858	2.95	↓
7	61.87	315669	8	60.19	322758		
8	53.55	126629	9	52.10	183063		
9	49.92	84095	10	49.77	69083	1.21	
10	48.29	271467	11	47.86	117733	2.30	↓
11	47.64	109949	12	47.36	107725		
12	45.47	99385	13	44.19	651354	6.55	↑
13	44.52	376551	14	43.59	397262		
14	42.81	737256	15	42.36	540015	1.36	
15	42.11	371686	16	40.92	230740	1.61	
16	41.05	364458	17	40.37	602009	1.65	
17	40.31	761303					
18	39.41	760747					
19	37.50	342496	18	37.56	324148		
20	36.05	57407	19	35.97	16402	3.5	↓
21	32.51	634813	20	33.15	499427	1.27	
22	30.34	152205	21	30.15	210446	1.38	
23	28.75	18070	22	28.81	81176	4.49	↑
24	28.32	12232	23	28.02	43646		
25	27.13	118428	24	27.19	49067	2.41	↓
26	25.05	96883	25	26.10	63245		
27	25	76867	26	25.70	56851		
28	25	83400	27	25	13344	6.25	↓
29	25	441464	28	25	22657	19.48	↓
30	25	57963	29	25	38225		
31	25	29329	30	25	406297	13.85	↑
32	25	35167	31	25	81037		
33	25	78118	32	25	72002		
34	25	48372	33	25	226014	4.67	↑
35	25	32526	34	25	73253		
36	25	57407	35	25	130938		
37	25	35723	36	25	125239	3.505	↑
38	25	29329	37	25	89238	3.042	↑
39	25	16263	38	25	38503		
40	25	7089	39	25	31831		
41	25	2780	40	25	17931		
42	25	8062	41	25	13205		
			42	25	11676		
			43	25	28078		

Table 4.2.a: Table showing the densitometry analysis of C306 and Chhotilerma, where the protein band intensity is compared.

	C306			Kalyansona			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
1	135.29	614241	1	139.33	199882		
2	111.69	218925	2	111.69	40171	5.44	↓
			3	104.52	42534		
3	94.40	9869	4	93.77	21267	2.15	↑
			5	90.65	10842		
4	81.07	646489	6	78.63	967857	1.49	
5	77.84	86180	7	75.76	146506	1.7	
6	71.47	104528	8	67.65	42117	2.48	↓
7	61.87	315669	9	58.16	460507		
8	53.55	126629	10	50	291066	2.29	↑
9	49.92	84095	11	47.72	343886	4.08	↑
10	48.29	271467					
11	47.64	109949	12	47.00	91601	1.20	
12	45.47	99385	13	45.06	218647	2.2	↑
13	44.52	376551	14	44.06	432012		
14	42.81	737256	15	42.36	384613		
15	42.11	371686					
16	41.05	364458	16	41.79	672065	1.84	
17	40.31	761303	17	40.55	383640		
18	39.41	760747	18	39.65	981479	1.29	
			19	38.88	952567		
19	37.50	342496	20	37.33	561699	1.64	
20	36.05	57407	21	36.13	140112	2.44	↑
21	32.51	634813	22	31.48	937694	1.47	
22	30.34	152205	23	29.82	625361	4.10	↑
23	28.75	18070	24	28.69	412830	22.84	↑
24	28.32	12232					
25	27.13	118428	25	27.36	297738	2.51	↑
26	25.05	96883	26	25.27	66720	1.45	
27	25	76867	27	25	248115	3.22	
28	25	83400	28	25	507906	6.09	↑
29	25	441464	29	25	375578	0.8508	
30	25	57963	30	25	71307	1.230	
31	25	29329	31	25	13900	2.11	↓
32	25	35167	32	25	21962	1.60	
33	25	78118	33	25	11398	6.85	↓
34	25	48372	34	25	18070	2.67	↓
35	25	32526	35	25	16541	1.96	
36	25	57407	36	25	11259	5.09	↓
37	25	35723	37	25	11676	3.05	↓
38	25	29329	38	25	10008	2.93	↓
39	25	16263					
40	25	7089					

Table 4.2.b: Table showing the densitometry analysis of C306 and Kalyansona, where the protein band intensity is compared

	C306			Lerma rajo			
Band No.	Mol. Wt. (KDa)	Intensity	Band No.	Mol.Wt. (KDa)	Intensity	Fold change	Up/down
1	135.29	614241	1	135.29	386698	1.58	
			2	125.67	182368		
2	111.69	218925					
3	94.40	9869	3	89.43	52125	5.28	↑
			4	87.33	29051		
4	81.07	646489					
5	77.84	86180	5	77.31	815652	9.46	↑
6	71.47	104528	6	73.46	141085	1.34	
7	61.87	315669	7	65.36	48094		
8	53.55	126629	8	55.81	322202		
9	49.92	84095	9	49.69	189040	2.24	↑
10	48.29	271467	10	48.80	44619	6.08	↓
11	47.64	109949	11	47.29	236022	2.14	↑
12	45.47	99385	12	46.51	55461	1.79	
13	44.52	376551	13	43.40	681656		
14	42.81	737256	14	42.81	362095	2.03	↓
15	42.11	371686					
16	41.05	364458	15	41.61	404629	1.11	
17	40.31	761303	16	40.13	234215	3.25	↓
18	39.41	760747	17	39.23	1080308	1.42	
19	37.50	342496					
20	36.05	57407	18	36.92	337631	5.88	↑
				35.43	19043		
21	32.51	634813	19	33.58	19877		
22	30.34	152205	20	31.68	755604	4.96	↑
23	28.75	18070	21	29.89	134413		
24	28.32	12232	22	28.14	129131		
25	27.13	118428	23	27.01	169163	1.42	
26	25.05	96883	24	25.27	76728	1.26	
27	25	76867	25	25	71029	1.08	
28	25	83400	26	25	151093	1.81	
29	25	441464	27	25	302186	1.46	
30	25	57963	28	25	26688	2.17	↓
31	25	29329	29	25	85485	2.91	
32	25	35167	30	25	83956	2.38	
33	25	78118	31	25	57963	1.34	
34	25	48372	32	25	56434	1.16	
35	25	32526	33	25	6533	4.97	↓
36	25	57407	34	25	1946	29.5	↓
37	25	35723	35	25	10008	3.56	↓
38	25	29329	36	25	15151	1.93	
39	25	16263	37	25	16958	1.04	
40	25	7089	38	25	4031	1.75	
41	25	2780	39	25	12649	4.55	↑
42	25	8062	40	25	6394	0.79	
			41	25	8062		
			42	25	8896		
			43				

Table 4.2.c: Table showing the densitometry analysis of C306 and Lermarajo, where the protein band intensity is compared.

	C306			Sonalika			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
1	135.29	614241	1	134.29	384752	1.59	
2	111.69	218925					
			2	105.29	24325		
			3	98.65	26827		
3	94.40	9869	4	95.69	11259	1.14	
			5	87.63	58241		
4	81.07	646489					
5	77.84	86180	6	76.02	813706		
6	71.47	104528	7	71.47	144560	1.38	
7	61.87	315669	8	63.16	122042		
8	53.55	126629	9	53.92	319978	2.52	↑
9	49.92	84095	10	49.25	215450	2.56	↑
10	48.29	271467	11	48.44	40449		
11	47.64	109949	12	46.86	232130	2.11	↑
12	45.47	99385	13	46.23	68388		
13	44.52	376551	14	44.32	53376	7.05	↓
14	42.81	737256	15	43.20	479967		
15	42.11	371686					
16	41.05	364458	16	41.42	807729	2.21	↑
17	40.31	761303					
18	39.41	760747	17	38.82	1264761	1.66	
19	37.50	342496					
20	36.05	57407	18	36.36	327484	5.70	↑
			19	35.06	57685		
21	32.51	634813	20	31.95	351809	1.80	
22	30.34	152205	21	29.13	81871	1.85	
23	28.75	18070	22	28.08	28217	1.56	
24	28.32	12232	23	26.49	142753	11.67	↑
25	27.13	118428	24	25	46426	2.55	↓
26	25.05	96883	25	25	98690	1.01	
27	25	76867	26	25	113285	1.47	
28	25	83400	27	25	172638	2.07	↑
29	25	441464	28	25	53376	8.27	↓
30	25	57963	29	25	16124	3.59	↓
31	25	29329	30	25	112034	3.81	↑
32	25	35167	31	25	20016	1.75	
33	25	78118	32	25	94381	1.20	
34	25	48372	33	25	30024	1.61	
35	25	32526	34	25	44758	1.37	
36	25	57407	35	25	18487	3.10	↓
37	25	35723	36	25	9730	3.67	↓
38	25	29329	37	25	2085	14.06	↓
39	25	16263	38	25	6116	2.65	↓
40	25	7089	39	25	15151	2.13	↑

Table 4.2.d: Table showing the densitometry analysis of C306 and Sonalika, where the protein band intensity is compared.

	C306			HD2009			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
1	135.29	614241	1	131.35	527783		
			2	122.92	227682		
2	111.69	218925					
			3	104.52	45592		
			4	98.65	23491		
3	94.40	9869	5	95.04	8618	1.145	
			6	87.93	16402		
4	81.07	646489	7	84.14	21267	30.39	↓
5	77.84	86180	8	76.02	944366	10.95	↑
6	71.47	104528	9	70.50	136915	1.30	
7	61.87	315669	10	64.034	25854	12.20	↓
			11	55.049	267158		
8	53.55	126629	12	52.82	212114	1.67	
9	49.92	84095	13	49.17	298850	3.55	↑
10	48.29	271467					
11	47.64	109949	14	46.86	329847	3	↑
12	45.47	99385	15	46.16	95771	1.037	
13	44.52	376551	16	44.19	343747	1.095	
14	42.81	737256	17	42.75	885569	1.201	
15	42.11	371686					
16	41.05	364458					
17	40.31	761303	18	40.92	396289	1.921	
18	39.41	760747	19	39.83	586858	1.29	
			20	39.06	792856		
19	37.50	342496	21	38.13	1192064	3.48	↑
20	36.05	57407	22	36.52	426452	7.42	↑
			23	34.60	55322		
21	32.51	634813	24	31.82	235188	2.69	↓
22	30.34	152205	25	31.14	223929	1.471	
23	28.75	18070	26	29.00	206832	11.44	↑
24	28.32	12232					
25	27.13	118428	27	27.84	159294	1.34	
			28	26.55	195156		
26	25.05	96883	29	26.04	78396	1.23	
27	25	76867	30	25	137749	1.79	
28	25	83400	31	25	9313	8.95	↓
29	25	441464	32	25	382250	1.154	
30	25	57963	33	25	459395	7.92	↑
31	25	29329	34	25	96188	3.27	
32	25	35167	35	25	524864	14.92	↑
33	25	78118	36	25	162074	2.07	↑
34	25	48372	37	25	105223	2.175	↑
35	25	32526	38	25	47955	1.474	
36	25	57407	39	25	14873	3.85	↓
37	25	35723	40	25	7923	4.50	↓
38	25	29329	41	25	7228	4.057	↓
39	25	16263	42	25	10564	1.53	
40	25	7089					

Table 4.2.e: Table showing the densitometry analysis of C306 and HD 2009, where the protein band intensity is compared.

	C306			HD2189			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
1	135.29	614241	1	136.29	1883867	3.066	↑
2	111.69	218925					
			2	109.24	241999		
			3	98.98	6672		
3	94.40	9869	4	95.37	9313	1.059	
			5	92.51	12649		
			6	87.63	53932		
4	81.07	646489	7	85.29	26549	24.35	↓
5	77.84	86180	8	76.53	1296314	15.04	↑
6	71.47	104528	9	70.98	176113	1.68	
7	61.87	315669	10	63.16	51986	6.072	↓
			11	55.42	452862		
8	53.55	126629	12	52.82	416722	3.29	↑
9	49.92	84095					
10	48.29	271467	13	48.95	628280	2.31	↑
11	47.64	109949	14	47.00	453279	4.12	↑
12	45.47	99385					
13	44.52	376551	15	44.39	149703	2.51	↓
14	42.81	737256	16	43.40	710429	1.03	
15	42.11	371686					
16	41.05	364458	17	41.48	3054108	8.37	↑
17	40.31	761303					
18	39.41	760747	18	39.17	1734164	2.27	
19	37.50	342496	19	38.24	1663830	4.85	
20	36.05	57407	20	36.68	594920	10.36	↑
21	32.51	634813	21	35.21	183758		
22	30.34	152205	22	30.41	1250861	8.21	↑
23	28.75	18070	23	28.88	800640	44.30	↑
24	28.32	12232					
25	27.13	118428	24	27.66	371408	3.136	
26	25.05	96883	25	26.32	255343	2.63	
27	25	76867	26	25	101748	1.32	
28	25	83400	27	25	64357	1.29	
29	25	441464	28	25	1951282	4.42	↑
30	25	57963	29	25	813150	14.02	↑
31	25	29329	30	25	152622	5.20	↑
32	25	35167	31	25	148174	4.21	↑
33	25	78118	32	25	7367	10.60	↓
34	25	48372	33	25	13900	3.48	↓
35	25	32526	34	25	17792	1.82	
36	25	57407	35	25	7923	7.24	↓
37	25	35723	36	25	2919	12.23	↓
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.f: Table showing the densitometry analysis of C306 and HD 2189, where the protein band intensity is compared.

	C306			UP262			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
1	135.29	614241					
2	111.69	218925					
			1	107.65	685826		
			2	98.65	179449		
3	94.40	9869	3	96.34	65886	6.67	↑
			4	88.22	36418		
4	81.07	646489					
5	77.84	86180	5	76.79	1066825	12.37	↑
6	71.47	104528	6	70.50	135664	1.29	
7	61.87	315669	7	64.47	327762	1.03	
			8	55.42	224485		
8	53.55	126629	9	53.19	220037	1.73	
9	49.92	84095	10	49.17	386281	4.59	↑
10	48.29	271467					
11	47.64	109949	11	47.14	272718	2.48	↑
			12	46.37	82288		
12	45.47	99385	13	45.40	31831	3.12	↓
13	44.52	376551	14	44.46	119679	3.14	↓
14	42.81	737256	15	43.33	826494	1.12	
15	42.11	371686	16	41.61	986205	2.65	↑
16	41.05	364458	17	41.05	508045	1.39	
17	40.31	761303					
18	39.41	760747	18	39.17	995240	1.30	
19	37.50	342496	19	38.30	1416688	4.13	↑
20	36.05	57407	20	36.60	302742	5.27	↑
21	32.51	634813	21	34.31	104667		
22	30.34	152205	22	31.48	630782	4.14	↑
23	28.75	18070	23	28.63	490948	27.16	↑
24	28.32	12232					
25	27.132	118428	24	26.44	177086	1.49	
26	25.05	96883	25	25	72280	1.34	
27	25	76867	26	25	50735	1.51	
28	25	83400	27	25	55183	1.51	
29	25	441464	28	25	1784760	4.04	↑
30	25	57963	29	25	113007	1.94	
31	25	29329	30	25	515829	17.58	↑
32	25	35167	31	25	260625	7.41	↑
33	25	78118	32	25	235188	3.01	↑
34	25	48372	33	25	4587	10.54	↓
35	25	32526	34	25	41839	1.28	
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.g: Table showing the densitometry analysis of C306 and UP 262, where the protein band intensity is compared.

	C306			VL421			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	152028		
			2	193.649175	42024		
			3	175.962043	34505		
1	135.29	614241					
2	111.69	218925					
3	94.40	9869	4	93.06048	283559	28.73	↑
4	81.07	646489					
5	77.84	86180	5	78.683627	60461	1.42	
6	71.47	104528	6	69.314	32239	3.24	↓
7	61.87	315669	7	58.53	147187	2.14	↓
8	53.55	126629	8	51.13	96923	1.30	
9	49.92	84095					
10	48.29	271467					
11	47.64	109949	9	47.91	139359	1.26	
12	45.47	99385					
13	44.52	376551					
14	42.81	737256	10	43.99	180559	4.08	↓
15	42.11	371686	11	42.15	231750	1.60	
16	41.05	364458					
17	40.31	761303					
18	39.41	760747	12	39.69	228763	3.32	↓
19	37.50	342496	13	38.90	260075	1.31	
20	36.055	57407	14	36.87	144097	2.51	↑
21	32.51	634813	15	35.50	33784	18.79	↓
22	30.34	152205	16	31.80	292829	1.92	
23	28.75	18070	17	29.89	99395	5.50	↑
24	28.32	12232	18	28.29	76838	6.28	↑
25	27.13	118428	19	26.87	82606	1.43	
26	25.05	96883	20	25	38522	2.51	↓
27	25	76867	21	25	218051	2.83	↑
28	25	83400	22	25	80031	1.042	
29	25	441464	23	25	5150	85.72	↓
30	25	57963	24	25	13390	4.32	↓
31	25	29329	25	25	4223	6.94	↓
32	25	35167	26	25	5459	6.44	↓
33	25	78118	27	25	5356	14.58	↓
34	25	48372	28	25	4738	10.20	↓
35	25	32526	29	25	10094	3.22	↓
36	25	57407	30	25	195803	3.41	↑
37	25	35723	31	25	735626	20.59	↑
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.h: Table showing the densitometry analysis of C306 and UP 262, where the protein band intensity is compared.

	C306			WH157			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	327952		
			2	181.67	183958		
1	135.29	614241	3	136.72	33166	18.52	↓
2	111.69	218925					
3	94.40	9869					
4	81.07	646489	4	84.55	272435	2.37	↓
5	77.84	86180					
6	71.47	104528					
7	61.87	315669	5	66.26	19673	16.04	↓
8	53.55	126629	6	56.59	95172	1.33	
9	49.92	84095	7	49.74	92185	1.09	
10	48.29	271467					
11	47.64	109949	8	47.433	99292	1.10	
12	45.47	99385					
13	44.52	376551					
14	42.81	737256	9	43.44	325583	2.26	↓
15	42.11	371686					
16	41.05	364458	10	41.84	206927	1.76	
17	40.31	761303	11	41.21	177366	4.29	↓
18	39.41	760747	12	39.39	342578	2.22	↓
19	37.50	342496	13	38.51	230102	1.48	
20	36.05	57407	14	36.62	228763	3.98	↑
21	32.51	634813	15	32.02	182619	3.47	↓
22	30.34	152205	16	28.39	64890	2.34	↓
23	28.75	18070					
24	28.32	12232					
25	27.13	118428	17	26.68	48410	2.44	↓
26	25.05	96883	18	25	23999	4.036	↓
27	25	76867	19	25	187151	2.43	↑
28	25	83400	20	25	81679	1.021	
29	25	441464	21	25	9682	45.59	↓
30	25	57963	22	25	4223	13.72	↓
31	25	29329	23	25	2266	12.94	↓
32	25	35167	24	25	4326	8.12	↓
33	25	78118	25	25	3811	20.49	↓
34	25	48372	26	25	612850	12.66	↑
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.i: Table showing the densitometry analysis of C306 and WH 157, where the protein band intensity is compared.

	C306			WL711			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	137608		
			2	250	161916		
			3	227.16	138638		
			4	193.64	120716		
			5	157.35	84872		
1	135.29	614241					
2	111.69	218925					
			6	123.18	5356		
3	94.40	9869					
4	81.07	646489	7	80.59	256161	2.52	↓
5	77.84	86180					
6	71.47	104528	8	70.89	19570	5.341	↓
7	61.87	315669	9	63.34	7107	44.41	↓
8	53.55	126629	10	54.71	111858	1.13	
9	49.92	84095	11	49.37	57165	1.47	
10	48.29	271467					
11	47.64	109949	12	47.07	95172		
12	45.47	99385					
13	44.52	376551					
14	42.81	737256	13	43.22	165418	4.45	↓
15	42.11	371686					
16	41.05	364458	14	41.52	147187	2.47	↓
17	40.31	761303	15	40.90	183237	4.15	↓
18	39.41	760747	16	38.61	691130		
19	37.50	342496					
20	36.05	57407	17	36.24	206618	3.59	↓
21	32.51	634813					
22	30.34	152205	18	31.15	282838	1.85	
23	28.75	18070	19	28.98	130604	7.22	↑
24	28.32	12232					
25	27.13	118428	20	27.62	111755		
26	25.05	96883	21	26.50	80134	1.20	
27	25	76867	22	25	21218	3.62	↓
28	25	83400	23	25	254513	3.051	↑
29	25	441464	24	25	86520	5.10	↓
30	25	57963	25	25	13493	4.29	↓
31	25	29329	26	25	2781	10.54	↓
32	25	35167	27	25	10712	3.28	↓
33	25	78118	28	25	5047	15.47	↓
34	25	48372	29	25	15862	3.04	↓
35	25	32526	30	25	12566	2.58	↓
36	25	57407	31	25	520665	9.069	↑
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.j: Table showing the densitometry analysis of C306 and WL711, where the protein band intensity is compared.

	C306			HD2285			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	169126		
			2	250	250599		
			3	216.54	121643		
			4	190.58	155118		
1	135.29	614241					
2	111.69	218925					
3	94.40	9869					
4	81.07	646489					
5	77.84	86180	5	76.81	226085	2.62	↑
6	71.47	104528	6	69.31	33269	3.144	↓
7	61.87	315669	7	62.63	11227	28.11	↓
8	53.55	126629	8	53.49	105266	1.20	
9	49.92	84095	9	49.12	49955	1.68	
10	48.29	271467	10	48.39	23999	11.31	↑
11	47.64	109949					
12	45.47	99385	11	46.84	122570	1.23	
13	44.52	376551	12	44.10	51500	7.31	↓
14	42.817	737256	13	43.11	125454	5.87	↓
15	42.11	371686					
16	41.05	364458	14	41.00	395211	1.08	
17	40.315	761303					
18	39.41	760747	15	38.809	349582	2.17	↓
19	37.50	342496	16	37.94	384190	1.12	
20	36.05	57407	17	35.99	227218	3.957	↑
21	32.51	634813					
22	30.34	152205	18	31.36	191683	1.25	
23	28.75	18070	19	28.98	94966	5.25	↑
24	28.32	12232	20	27.71	59122	4.83	↑
25	27.132	118428	21	26.50	91670	1.29	
26	25.05	96883	22	25	27501	3.52	↓
27	25	76867	23	25	182413	2.37	↑
28	25	83400	24	25	21733	3.83	↓
29	25	441464	25	25	19776	22.32	↓
30	25	57963	26	25	80649	1.39	
31	25	29329	27	25	34196	1.16	
32	25	35167	28	25	11227	3.13	↓
33	25	78118	29	25	8961	8.71	↓
34	25	48372	30	25	64066	1.32	
35	25	32526	31	25	47586	1.46	
36	25	57407	32	25	376877	6.56	↑
37	25	35723					
38	25	29329					
39	25	16263					

Table 4.2.k: Table showing the densitometry analysis of C306 and HD 2285, where the protein band intensity is compared.

	C306			HD2329			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	121128		
			2	250	153058		
			3	220.02	146054		
			4	146.56	4635		
1	135.29	614241					
2	111.69	218925	5	117.60	15656	13.98	↓
3	94.40	9869					
4	81.07	646489					
5	77.84	86180	6	74.16	460410	5.342	↑
6	71.47	104528					
7	61.87	315669	7	60.55	141213	2.23	↓
8	53.55	126629	8	51.13	124115	1.02	
9	49.92	84095					
10	48.29	271467	9	48.39	172834	1.57	
11	47.64	109949					
12	45.47	99385	10	46.60	95893	1.03	
13	44.52	376551	11	43.88	95893	3.92	↓
14	42.81	737256	12	42.79	187460	3.93	↓
15	42.11	371686					
16	41.05	364458					
17	40.31	761303	13	40.80	428892	1.77	
18	39.41	760747	14	38.70	320742	2.37	↓
19	37.50	342496	15	37.27	864891	2.52	↑
20	36.05	57407	16	34.42	71173		
21	32.51	634813					
22	30.34	152205	17	29.38	647870	4.25	↑
23	28.75	18070	18	28.29	470813	26.05	↑
24	28.32	12232					
25	27.13	118428	19	26.77	450316	3.80	↑
26	25.05	96883	20	25	103927	1.072	
27	25	76867	21	25	814318	10.59	↑
28	25	83400	22	25	906503	10.86	↑
29	25	441464	23	25	246891	1.78	
30	25	57963	24	25	43878	1.32	
31	25	29329	25	25	16274	1.80	
32	25	35167	26	25	71173	2.02	↑
33	25	78118	27	25	75396	1.03	
34	25	48372	28	25	397786	8.22	↑
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.I: Table showing the densitometry analysis of C306 and HD 2329, where the protein band intensity is compared.

	C306			HI617			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	204043		
			2	238.30	224128		
			3	193.64	101867		
			4	154.86	103927		
1	135.29	614241					
2	111.69	218925	5	117.60	23896	9.16	↓
3	94.40	9869					
			6	88.70	230308		
4	81.07	646489					
5	77.84	86180	7	74.16	41509	2.07	↓
6	71.47	104528	8	67.01	16377	6.38	↓
7	61.87	315669	9	59.208	85799	3.67	↓
8	53.55	126629	10	50.566	42951	2.94	↓
9	49.92	84095					
10	48.29	271467	11	48.88	21115	12.85	↓
11	47.64	109949	12	47.79	42745	2.57	↓
12	45.47	99385	13	46.84	55517	1.79	
13	44.52	376551	14	43.77	50676	7.43	↓
14	42.81	737256	15	42.05	92391	7.97	↓
15	42.11	371686					
16	41.05	364458	16	41.31	171495	2.12	↓
17	40.31	761303					
18	39.41	760747	17	38.70	492134	1.54	
19	37.50	342496	18	37.09	156972	2.18	↓
20	36.05	57407					
21	32.51	634813	19	31.801	227527	2.79	↓
22	30.349	152205	20	29.18	45732	3.327	↓
23	28.75	18070					
24	28.32	12232					
25	27.13	118428	21	26.59	35020	3.38	↓
26	25.05	96883	22	25	14626	6.62	↓
27	25	76867	23	25	164182	2.13	↑
28	25	83400	24	25	7313	11.40	↓
29	25	441464	25	25	28840	15.30	↓
30	25	57963	26	25	43260	1.33	
31	25	29329	27	25	55620	1.89	
32	25	35167	28	25	16274	2.16	↓
33	25	78118	29	25	3708	21.06	↓
34	25	48372	30	25	30591	1.58	
35	25	32526	31	25	145539	4.47	↑
36	25	57407	32	25	614292	10.70	↑
37	25	35723					
38	25	29329					
39	25	16263					

Table 4.2.m: Table showing the densitometry analysis of C306 and HI 617, where the protein band intensity is compared.

	C306			HS240			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	193.64	220729		
1	135.29	614241					
2	111.69	218925					
3	94.40	9869					
4	81.07	646489	2	84.55	271714		
5	77.84	86180					
6	71.47	104528	3	73.32	30694	3.40	↓
7	61.87	315669	4	66.26	16377	19.27	↓
8	53.55	126629	5	55.96	103824	1.21	
9	49.92	84095	6	49.62	147702	1.75	
10	48.29	271467					
11	47.64	109949	7	47.43-‘	118244	1.07	
12	45.47	99385					
13	44.52	376551	8	44.66	56547	6.65	↓
14	42.817	737256	9	43.44	119480	6.17	↓
15	42.11	371686					
16	41.05	364458	10	41.31	518399	1.42	
17	40.31	761303					
18	39.41	760747	11	39.19	338355	2.24	↓
19	37.50	342496	12	38.22	306116	1.11	
20	36.05	57407	13	36.36	161813	2.81	↑
21	32.51	634813	14	34.77	10918	58.14	↓
22	30.34	152205	15	31.47	223201	1.46	
23	28.75	18070	16	29.08	25132	1.39	
24	28.326	12232					
25	27.13	118428	17	27.71	59946	1.97	
26	25.05	96883	18	26.41	75499	1.28	
27	25	76867	19	25	11433	6.72	↓
28	25	83400	20	25	61182	1.36	
29	25	441464	21	25	613674	1.39	
30	25	57963	22	25	435587	7.51	↑
31	25	29329	23	25	213828	7.29	↑
32	25	35167	24	25	65817	1.87	
33	25	78118	25	25	81885	1.04	
34	25	48372	26	25	110107	2.27	↑
35	25	32526	27	25	369049	11.34	↑
36	25	57407	28	25	358440	6.24	↑
37	25	35723					
38	25	29329					
39	25	16263					

Table 4.2.n: Table showing the densitometry analysis of C306 and HS 240, where the protein band intensity is compared.

		C306		HUW 206			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	1406148		
			2	206.41	1189254		
1	135.29	614241					
2	111.69	218925	3	110.39	32412	6.75	↓
3	94.40	9869	4	96.18	179931	18.23	↑
			5	87.61	454878		
4	81.07	646489					
5	77.84	86180	6	79.19	172383	2.00	↑
6	71.47	104528					
7	61.87	315669	7	58.21	372627	1.18	
8	53.55	126629					
9	49.92	84095					
10	48.29	271467					
11	47.64	109949					
12	45.47	99385					
13	44.52	376551					
14	42.81	737256					
15	42.11	371686					
16	41.05	364458					
17	40.31	761303					
18	39.41	760747	8	39.74	456654	1.66	
19	37.50	342496					
20	36.05	57407	9	36.20	412254	7.18	↑
			10	33.36	541347		
21	32.51	634813	11	32.64	409923	1.54	
22	30.34	152205	12	29.92	661893	4.34	↑
23	28.75	18070	13	28.18	459429	25.42	↑
24	28.32	12232					
25	27.13	118428	14	26.25	213564	1.80	
26	25.05	96883	15	24.83	616494	6.363	↑
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					
			16	23.566081	124764		
			17	22.606612	212121		
			18	21.972741	129759		

Table 4.2.o: Table showing the densitometry analysis of C306 and HUW 206, where the protein band intensity is compared.

	C306			LOK1			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	1111887		
			2	216.54	1289376		
1	135.29	614241					
2	111.69	218925	3	110.39	411477	1.87	
3	94.40	9869					
			4	87.61	304695		
4	81.07	646489					
5	77.844	86180	5	77.97	155400	1.80	
6	71.47	104528					
7	61.87	315669	6	59.70	123099	2.56	↓
8	53.55	126629					
9	49.92	84095					
10	48.29	271467					
11	47.64	109949					
12	45.47	99385					
13	44.52	376551					
14	42.81	737256					
15	42.11	371686					
16	41.05	364458					
17	40.31	761303					
18	39.41	760747					
19	37.50	342496					
20	36.05	57407	7	36.00	745476	12.98	↑
21	32.51	634813	8	33.18	899655	1.41	
22	30.34	152205	9	29.92	951270	6.24	↑
23	28.75	18070					
24	28.32	12232					
25	27.13	118428					
26	25.05	96883	10	26.54	294816	3.042	↑
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					
			11	23.669417	882006		

Table 4.2.p: Table showing the densitometry analysis of C306 and LOK1, where the protein band intensity is compared.

	C306			VL616			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	1489953		
			2	193.64	428349		
			3	141.35	67488		
1	135.29	614241					
2	111.69	218925	4	109.30	363081	1.65	
3	94.40	9869	5	96.18	8436	1.16	
			6	87.61	129204		
4	81.07	646489	7	79.81	91020	7.10	↓
5	77.84	86180					
6	71.47	104528					
7	61.87	315669	8	58.21	190698	1.65	
8	53.55	126629					
9	49.92	84095					
10	48.29	271467					
11	47.64	109949					
12	45.47	99385					
13	44.52	376551					
14	42.81	737256					
15	42.11	371686					
16	41.05	364458					
17	40.31	761303	9	40.32	261183	2.91	↓
18	39.41	760747					
19	37.50	342496					
20	36.05	57407	10	36.40	304695	5.30	↑
21	32.51	634813	11	33.54	469641	1.35	
22	30.34	152205	12	30.08	846375	5.56	↑
23	28.75	18070	13	28.49	464646		
24	28.32	12232					
25	27.13	118428	14	26.54	367299	3.10	↑
26	25.05	96883	15	23.72	331335	3.41	↑
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.q: Table showing the densitometry analysis of C306 and VL616, where the protein band intensity is compared.

	C306			HS295			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	223.56	979797		
			2	148.52	118659		
1	135.29	614241					
2	111.69	218925	3	110.39	392496	1.79	
3	94.40	9869					
			4	86.93	198135		
4	81.07	646489	5	79.81	105894	6.105	↓
5	77.84	86180					
6	71.47	104528					
7	61.87	315669	6	59.70	154401	2.04	↓
8	53.55	126629	7	50	166056	1.31	
9	49.92	84095					
10	48.29	271467					
11	47.64	109949					
12	45.47	99385					
13	44.52	376551					
14	42.81	737256					
15	42.11	371686					
16	41.05	364458					
17	40.31	761303	8	40.90	156732	0.205	
18	39.41	760747					
19	37.50	342496					
20	36.05	57407	9	36.40	160173	2.79	↑
21	32.51	634813	10	33.72	139194	4.56	↓
			11	31.76	256854		
22	30.34	152205	12	30.24	284826	1.87	
23	28.75	18070	13	28.49	558219		
24	28.32	12232					
25	27.13	118428	14	26.83	158175	1.33	
26	25.05	96883	15	25.41	19758	4.90	↓
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.r: Table showing the densitometry analysis of C306 and HS295, where the protein band intensity is compared.

	C306			PBW343			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	921300		
			2	250	386169		
			3	209.73	194583		
			4	181.67	182595		
			5	152.41	93129		
1	135.29	614241					
2	111.69	218925	6	112.60	323010	1.47	
			7	98.45	25641		
3	94.40	9869	8	89.68	168276	17.05096768	↑
4	81.07	646489	9	82.33	83694	7.72	↓
5	77.84	86180					
6	71.47	104528					
7	61.87	315669	10	62.80	217005	1.45	
8	53.55	126629					
9	49.92	84095					
10	48.29	271467					
11	47.64	109949					
12	45.47	99385					
13	44.52	376551					
14	42.81	737256					
15	42.11	371686	11	42.09	190476	1.95	
16	41.05	364458					
17	40.31	761303					
18	39.41	760747					
19	37.50	342496	12	37.53	243867	1.40	
20	36.05	57407	13	34.09	676989	11.79	↑
21	32.51	634813	14	31.08	406260	1.56	
22	30.34	152205	15	29.59	347430	2.28	↑
23	28.75	18070					
24	28.32	12232					
25	27.13	118428	16	27.42	438006	3.69	↑
26	25.05	96883	17	24.19	277389	2.86	↑
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.s: Table showing the densitometry analysis of C306 and PBW343, where the protein band intensity is compared.

	C306			RAJ3765			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	890331		
			2	250	733044		
			3	152.41	48174		
1	135.29	614241					
2	111.69	218925	4	117.14	361749	1.65	
			5	100.99	10878		
3	94.40	9869	6	91.09	196137	19.87	↑
4	81.07	646489	7	83.62	163614	3.95	↓
5	77.84	86180					
6	71.47	104528	8	71.29	292041	2.793	↑
7	61.87	315669					
8	53.55	126629					
9	49.92	84095					
10	48.29	271467					
11	47.64	109949					
12	45.47	99385					
13	44.52	376551	9	43.32	355755	1.05	
14	42.81	737256					
15	42.11	371686					
16	41.05	364458					
17	40.33	761303					
18	39.415139	760747	10	38.07	386946	1.96	
19	37.504753	342496					
20	36.055845	57407					
21	32.514081	634813	11	34.84	691419	1.08	
22	30.348369	152205	12	31.25	443889	2.916	↑
23	28.757277	18070	13	29.75	539349	29.84	↑
24	28.326914	12232					
25	27.132461	118428	14	27.72	356088	3.006	↑
26	25.053909	96883	15	24.140	285825	2.953	↑
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					
40	25	7089					

Table 4.2.t: Table showing the densitometry analysis of C306 and RAJ3765, where the protein band intensity is compared.

	C306			UP2338			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	695304		
			2	223.56	355422		
			3	157.35	29193		
1	135.29	614241					
			4	120.67	536352		
2	111.69	218925					
3	94.40	9869	5	96.18	175935	17.82	↑
			6	92.51	162837		
4	81.07	646489	7	83.62	221889	2.91	↓
5	77.84	86180	8	75.58	93684	1.08	
6	71.47	104528					
			9	66.07	69930		
7	61.87	315669					
8	53.55	126629					
9	49.92	84095					
10	48.29	271467					
11	47.64	109949					
12	45.47	99385	10	45.22	213675	2.14	↑
13	44.52	376551					
14	42.81	737256					
15	42.11	371686					
16	41.05	364458					
17	40.31	761303					
18	39.41	760747	11	39.74	354090	2.14	↓
19	37.50	342496					
20	36.05	57407	12	35.23	602397	10.49	↑
21	32.51	634813	13	31.76	286935	2.21	↓
22	30.34	152205	14	29.92	684648	4.49	↑
23	28.75	18070	15	28.02	254301	14.07	↑
24	28.32	12232					
25	27.13	118428	16	26.25	157176	1.32	
26	25.05	96883	17	24.14	432345	4.46	↑
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					

Table 4.2.u: Table showing the densitometry analysis of C306 and UP2338, where the protein band intensity is compared.

	C306			GW322			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	269619		
			2	238.30	320457		
			3	167.73	20091		
1	135.29	614241					
			4	125.54	308580		
2	111.69	218925	5	109.30	19980	10.95	↓
3	94.40	9869	6	89.68	93906	9.51	↑
4	81.07	646489					
5	77.84	86180	7	77.36	207015	2.40	↑
6	71.47	104528					
7	61.87	315669					
8	53.55	126629					
9	49.92	84095					
10	48.29	271467	8	48.58	97347	2.78	↓
11	47.64	109949					
12	45.47	99385					
13	44.52	376551	9	43.94	114774	3.28	↓
14	42.81	737256					
15	42.11	371686					
16	41.05	364458					
17	40.31	761303					
18	39.41	760747					
19	37.50	342496					
20	36.05	57407	10	36.40	125874	2.19	↑
21	32.51	634813	11	32.64	511044	1.24	
22	30.34	152205	12	31.08	365190	2.39	↑
23	28.75	18070	13	28.64	254190	14.06	↑
24	28.32	12232					
25	27.13	118428	14	27.12	54945	2.15	↓
26	25.05	96883	15	24.51	249972	2.58	↑
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					
38	25	29329					
39	25	16263					

Table 4.2.v: Table showing the densitometry analysis of C306 and GW322, where the protein band intensity is compared.

	C306			PBW502			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	14160		
			2	250	981406		
			3	220.78	557904		
1	135.29	614241					
			4	124.75	244968		
			5	118.47	235174		
2	111.69	218925	6	110.05	152692	1.43	
3	94.40	9869	7	90.18	252402	25.57	↑
4	81.07	646489	8	84.39	138768	4.65	↓
5	77.84	86180					
			9	73.41	171218		
6	71.47	104528	10	71.86	121540	1.16	
			11	65.58	714018		
7	61.87	315669	12	61.51	975860	3.09	↑
			13	57.35	705876		
			14	55.29	562506		
8	53.55	126629	15	52.65	382910	3.02	↑
9	49.92	84095	16	50.61	121894	1.44	
10	48.29	271467					
11	47.64	109949					
12	45.47	99385	17	46.67	299484	3.01	↑
13	44.52	376551	18	44.01	116466	3.23	↓
14	42.81	737256					
15	42.11	371686	19	41.92	86258	4.30	↓
16	41.05	364458	20	41.18	116348	3.13	↓
17	40.31	761303					
18	39.41	760747					
19	37.50	342496	21	37.95	96170	3.56	↓
20	36.05	57407	22	37	794376	13.83	↑
			23	37	376420		
			24	37	12272		
			25	37	8732		
			26	37	9676		
			27	37	21004		
21	32.51	634813					
22	30.34	152205					
23	28.75	18070					
24	28.32	12232					
25	27.13	118428					
26	25.05	96883					
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					
37	25	35723					

Table 4.2.w: Table showing the densitometry analysis of C306 and PBW502, where the protein band intensity is compared.

	C306			DBW39			
Band No.	**Mol. Wt. (KDa)**	**Intensity**	**Band No.**	**Mol. Wt. (KDa)**	**Intensity**	**Fold change**	**Up/down**
			1	250	1008900		
			2	250	452766		
			3	197.70	256414		
			4	160.72	68204		
			5	120.23	379724		
1	135.29	614241					
2	111.69	218925	6	112.51	57230	3.82	↓
			7	105.29	34692		
3	94.40	9869	8	92.20	257240	26.065	↑
4	81.07	646489	9	85.64	133222	4.85	↓
5	77.84	86180					
			10	73.86	132632		
6	71.47	104528	11	72.30	108442	1.037	
			12	65.78	1018104		
			13	62.27	471646		
7	61.87	315669	14	61.33	421378	1.33	
			15	57.52	869542		
			16	55.79	576666		
8	53.55	126629	17	53.143	407690	3.21	↑
9	49.92	84095	18	50.92	138060	1.64	
10	48.298	271467					
11	47.64	109949	19	47.149	417720	3.79	↑
12	45.47	99385					
13	44.52	376551	20	44.68	121894	3.08	↓
14	42.81	737256					
15	42.11	371686					
16	41.05	364458	21	41.39	97940	3.721	↓
17	40.31	761303					
18	39.41	760747	22	39.23	21830	34.84	↓
19	37.50	342496	23	37	608880	1.77	
20	36.05	57407	24	37	87202	1.51	
			25	37	122956		
			26	37	35282		
			27	37	6844		
			28	37	28438		
			29	37	5074		
			30	37	54162		
21	32.51	634813					
22	30.34	152205					
23	28.75	18070					
24	28.32	12232					
25	27.13	118428					
26	25.05	96883					
27	25	76867					
28	25	83400					
29	25	441464					
30	25	57963					
31	25	29329					
32	25	35167					
33	25	78118					
34	25	48372					
35	25	32526					
36	25	57407					

37	25	35723					
38	25	29329					

Table 4.2.x: Table showing the densitometry analysis of C306 and DBW39, where the protein band intensity is compared.

4.3 Analysis based on SDS-PAGE

Cluster analysis was carried out on the results of SDS-PAGE using computer software DendroUPGMA in order to study distance or similarity among the given wheat varieties. The result of cluster analysis is given in the dendrogram (**Figure 4.3**) by the procedure of "unweighted pair group method with arithmetic means"(UPGMA).In the present study dendrogram was calculated from Jaccard similarity coefficient un-weighted pair group method with averages constructed by gluten bands.Lermarajo and HS240 are outgroup (entirely different from the rest of the other varieties). The dendrogram revealed two major clads – clad1 and clad 2. Clad 1 is further distributed into 2 cluster. Also Clad 2 is further distributed into 2 cluster in which one cluster is again further distributed into 2 cluster. Varieties of clad 1 cluster 1 are WH157, HD2285 and clad1 cluster 2 are HI617, WL711 and HD2329. Varieties of clad 2 cluster 1 are UP262, HD2189, HD2009, Sonalika, C306 and Chotilerma and clad 2 cluster 2 are Kalyansona and HS295, VL421, PBW502, RAJ3765, VL616, PBW343, GW322, LOK1 and HUW206. Varieties shown in yellow color (UP262, HD2189, HD2009, Sonalika, C306 and Chotilerma) are similar as they all lack 250kDa, 150kDa and 20kDa bands. Kalyansona and HS295 (in green color) are different from the varieties in subcluster in yellow color because they only lack 250kDa band. VL421 and PBW502 are more similar to each other than to DBW39 (in blue color). Varieties in purple subcluster – RAJ3765, VL616 and PBW343 (having all the mol.wt bands present) and GW322, LOK1 and HUW206 are different from the other varieties in purple subcluster because they lack only 150kDa mol.wt band (Shuaib *et al.,*2010).The clustering of the varieties in the given dendrogram is purely on the basis of presence of bands of same mol.wt in them (except in two).Varieties in the yellow and purple color are clustered according to their origin also. Yellow color contains the oldest variety from 1965-1979, whereas purple color contains the newest variety from 1981-2002.

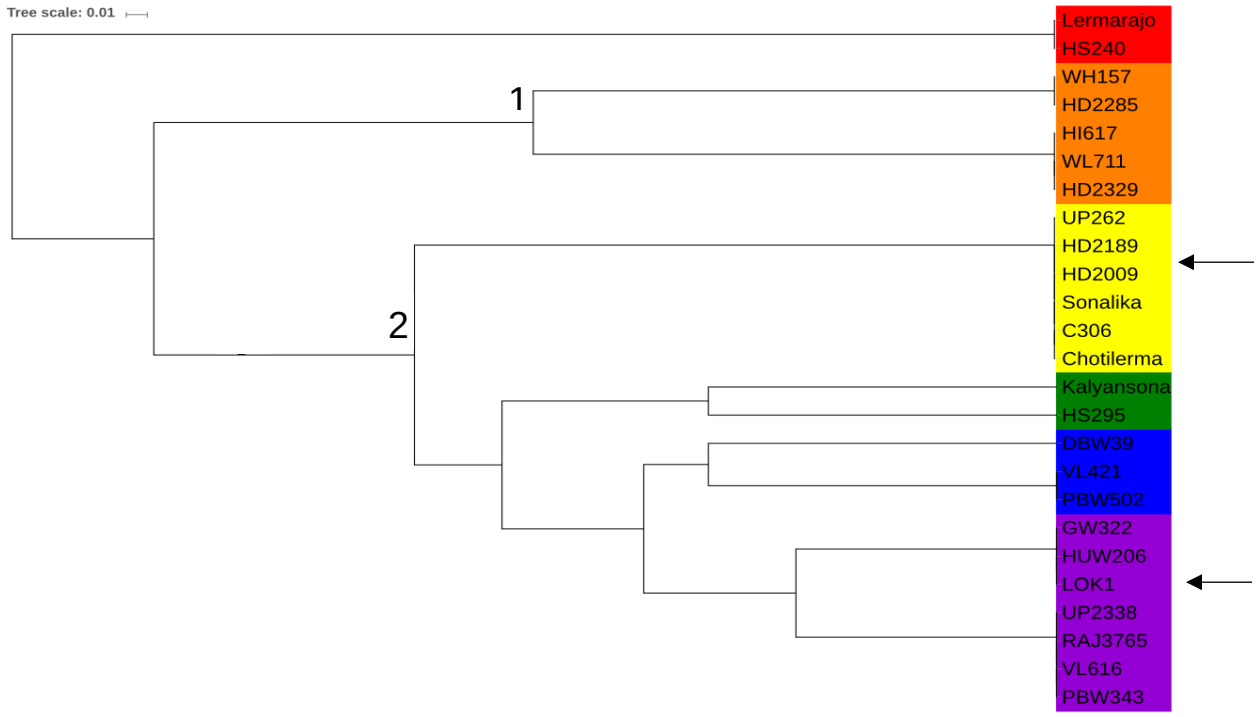

Figure 4.3 Dendrogram of 25 different wheat varieties based on SDS-PAGE (UPGMA).

4.4 Multiple Sequence Alignment

The α-gliadin gene name, sequence length and their accession number are summarized in **Table 4.3** Multiple sequence alignment analysis indicated numerous fully conserved residues in α-gliadin protein among the 15 different α- gliadin genes of *T.aestivum* as shown in **Figure 4.4.a** and maximum variation was observed in the region starting from amino acid no 222 -259 as shown in **Figure 4.4.b.** In the variable region glutamine, proline and serine were majorly found.

S.No	α-gliadin gene name	Accession number	Length(amino acids)
1.	Gli-G3	A5JSA6	307
2.	Gli-G5	A5JSA8	288
3.	Gli-G2	A5JSA5	285
4.	Gli-G4	A5JSA7	289
5.	Gli-G1	A5JSA4	313
6.	Gli-G6	A5JSA9	312
7.	Gli-G7	A5JSB0	285
8.	Gli-Z1	Q1WA39	306
9.	Gli-Z2	A5JSB2	290
10.	Gli-Z3	A5JSB3	287
11.	Gli-Z4	A5JSB4	287
12.	Gli-Z5	A5JSB5	291
13.	Gli-Z6	A5JSB6	291
14.	Gli-Z7	A5JSB7	286
15.	Gli-Z8	A5JSB8	308

Table 4.3. α-Gliadin protein length and their accession number

```
CLUSTAL O(1.2.4) multiple sequence alignment


A5JSA6      MKTFLILALLAIVATTATIAVRVPVPQLQPQNPSQQQPQEQVPLVQQQQFPGQQQPFPPQ        60
Q1WA39      MKTFLILALLAIVATTATIAVRVPVPQLQPQNPSQQQPQEQVPLVQQQQFPGQQQPFPPQ        60
A5JSA8      MKTFLILALLAIVATTATTAVRVPVPQLQLQNPSQQQPQEQVPLVQEQQFPGQQQPFPPQ        60
A5JSB5      MKTFLILALLAIVATTATTAVRVPVPQLQLQNPSQQQPQEQVPLVQEQQFPGQQQPFPPQ        60
A5JSB4      MKTFLILVLLAIVATTATTAVRFPVPQLQPQNPSQQQPQEQVPLVQQQQFLGQQEQFPPQ        60
A5JSB3      MKTFLILALLAIVATTATTAVRVPVPQLQPQNPSQQQPQEQVPLVQQQQFLGQQQPFPPQ        60
A5JSB2      MKTFLILALLAIVATTATTAVRVPVPQLQPQNPSQQQPQEQVPLVQQQQFPGQQQQFPPQ        60
A5JSB0      MKTFLILALLAIVATTATTAVRVPVPQLQPQNPSQQQPQEQVPLVQ-QQFLGQQQPFPPQ        59
A5JSA5      MKTFLILVLLAIVATTATTAVRFPVPQLQPQNPSQQQPQEQVPLVQQQQFLGQQQPFPPQ        60
A5JSB6      MKTFLILALLAIVATTATTAVRVPVPQSQPQNPSQQQPQEQVPLVQQQQFLGQQQPFPPQ        60
A5JSA7      MKTFLILALLAIVATTATIAVRVPVPQPQLQNPSQPQPQGQVPLVQQQQFPGQQQQFPPQ        60
A5JSB7      MKTFLILALLAIVATTATIAVRVPVPQLQPQNPSQQQPQKQVPLVQQQQFPGQQQPFPPQ        60
A5JSA4      MKTFLILALLAIVATTATTAVRVPVPQLQPQNPSQQQPQEQVPLVQQQQFPGQQQQFPPQ        60
A5JSA9      MKTFLILALLAIVATTATIAVRVPVPQLQPQNPSQQQPQEQVPLVQQQQFPGQQQPFPPQ        60
A5JSB8      MKTFLILALLAIVATTATTAVRVPVPQLQPQNPSQQQPQEQVPLVQQQQFPGQQQQFPPQ        60
            *******.********** ***.**** * ***** *** ****** *** ***: ****


  A5JSA6      QPYPQPQPFPSQQPYLQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPFRPQQPYPQSQPQ        120
  Q1WA39      QPYPQPQPFPSQQPYLQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPFRPQQPYPQSQPQ        120
  A5JSA8      QPYPQPQPFPSQQPYLQLQPFPQPQ--------------LPYPQPQPFRPQQPYPQPQPQ        106
  A5JSB5      QPYPQPQPFPSQQPYLQLQPFPQP---------------QLPYPQPQPFRPQQPYPQPQPQ        106
  A5JSB4      QPYPHQQPFPSQQPYPQPQPFPP----------------QLPYPQTQPFPPQQPYPQPQPQ        105
  A5JSB3      QPYPQPQPFPSQQPYLQLQPFPQP---------------QLPYSQPQPFRPQQPYPQPQPQ        106
  A5JSB2      QPYPQPQPFPSQQPYLQLQPFPQPQPF---------PPQLPYPQPQSFPPQQPYPQQQPQ        111
  A5JSB0      QPYPQPQPFPSQQPYLQLQPFLQP---------------QLPYSQPQPFRPQQPYPQPQPQ        105
  A5JSA5      QPYPQPQPFPSQLPYLQLQPFPQP---------------QLPYSQPQPFRPQQPYPQPQPQ        106
  A5JSB6      QPYPQPQPFPSQQPYLQLQPFPRPQPF---------PPQLPYPQTQPFRPQQPYPQPQPQ        111
  A5JSA7      QPYPQPQPFPSQQPYLQLQPFPQPQLPYP-------QPHLPYPQPQPFRPQQPYPQPQPQ        113
  A5JSB7      QPYPQLQPFPSQQPYMQLQPFPQPQLPYP-------QPQLPYPQPQPFRPQQSYPQPQPQ        113
  A5JSA4      QPYPQPQPFPSQQPYLQLQPFPQPQPF---------PPQLPYPQPQSFPPQQPYPQQQPQ        111
  A5JSA9      QPYPQPQPFPSQQPYLQLQPFPQPQPF---------PPQLPYPQPQSFPPQQPYPQQQPQ        111
  A5JSB8      QPYPQPQPFPSQQPYLQLQPFPQPQPF---------PPQLPYPQPHPFPPQQPYPQQQPQ        111
              ****: ****** ** * ***                  *** * : * *** *** ***


  A5JSA6      YSQPQQPISQQQQQQQQQQ-KQQQQQQQQQILQQILQQQLIPCRDVVLQQHSIAYGSSQV        179
  Q1WA39      YSQPQQPISQQQQQQQQQQQQKQQQQQQQILQQILQQQLIPCRDVVLQQHSIAYGSSQV        180
  A5JSA8      YPQPQQPISQQQQQQ---------QQQQQQILQQILQQQLIPCRDVVLQQHNIAHGSSQV        157
  A5JSB5      YSQPQQPISQQQQQQQQ------QQQQQQQILQQILQQQLIPCRDVVLQQHNIAHGSSQV        160
  A5JSB4      YPQPQQPISQQQAQQ---------QQQQQQILQQILQQQLIPCRDVVLQQHNIAHASSHV        156
  A5JSB3      YSQPQQPISQQQQQQQQQ--QQQQQQQEQQILQQILQQQLIPCMDVVLQQHNLAHGRSQV        164
  A5JSB2      YLQPQQPISQQQAQQQQ----QQQQQQEQQILQQILQQQLIPCMDVVLQQHNIAHGRSQV        167
  A5JSB0      YSQPQQPISQQQQQQQQ---QQQQQQQQQQIIQQILQQQLIPCMDVVLQQHNIVHGKSQV        162
  A5JSA5      YSQPQQPISQQQQQQQQ---Q-QQQQQQQQILQQILQQQLIPCMDVVLQQHNIAHGRSQV        162
  A5JSB6      YSQPQQPISQQQQQQQQ---QQQQQQQEQQILQQILQQQLIPCMEVVLQQHNIAHGRSQV        168
  A5JSA7      YSQPQQPISQQQQQQ--------QQQQQQQILQQILQQQLIPCRDVVLQQHNIAHASSQV        165
  A5JSB7      YSQPQQPISQQQQQ-----------QQQQQILQQILQQQLIPCRDVVLQQHSIAHGSSQV        162
  A5JSA4      YLQPQQPISQQQAQQQQQ--QQQQQQQQQQILQQILQQQLIPCRDVVLQQHNIAHASSQV        169
  A5JSA9      YLQPQQPISQQQAQQQQQ--Q-QQQQQQQQILQQILQQQLIPCRDVVLQQHNIAHASSQV        168
  A5JSB8      YLQPQQPISQQQAQQQQQ------QQQQQQILQQILQQQLIPCRDVVLQQHNIAHASSQV        165
              * ********** *           **:***:*********** :******.::.: *:*


A5JSA6      LQQSTYQLVRQLCCQQLWQIPEQSRCQAIHNVVHAIILHQQQQQQ--------------Q        225
Q1WA39      LQQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAIILHQQQQQQ--------------        225
A5JSA8      LQESTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAIILHQQHHHHQ-----------QQQ        206
A5JSB5      LQESTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAIILHQQHHHHQ-----------QQQ        209
A5JSB4      LQQSSYQQLQQLCCQQLFQIPEQSRCQAIHNVVHAIILHQQHHHHQ-----------QQQ        205
A5JSB3      LQQSTYQLLQELCCQHLWQIPEQSQCQAIHNVVHAIILHQQQKQ----------------        208
A5JSB2      LQQSTYQLLQELCCQHLWQIPEQSQCQAIHNVVHAIILHQQQKQ----------------        211
A5JSB0      LQQSTYQLLQELCCQHLWQIPEQSQCQAIHNVVHAIILHQQQKQ----------------        206
A5JSA5      LQQSTYQLLQELCCQHLWQIPEQSQCQAIHNVVHAIILHQQQKQ----------------        206
A5JSB6      LQQSTYQLLQELCCQHLWQIPEQSQCQAIHNVVHAIILHQQQKQ----------------        212
A5JSA7      LQQSTYQLLQQLCCQQLWQIPEQSRCQAIHNVVHAIILHQQQR----------------        208
A5JSB7      LQQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAIILHQQQQQ----------------        206
A5JSA4      LQQSTYQLLQQLCCQQLLQIPEQSRCQAIHNVAHAIIMHQQQQQQLQQQQQQQLQQQQQQ        229
A5JSA9      LQQSTYQLLQQLCCQQLLQIPEQSRCQAIHNVAHAIIMHQQQQQQQEQQQQLQQQQQQQL        228
A5JSB8      LQQSTYQLLQQWCCQQLLQIPEQSRCQAIHNVAHAIIMHQQQQQ-QEQQQQLQQQQQQQL        224
            **:*:** ::: ***:* ******:*******.****:***::


A5JSA6      QQQQKQPLSQVSFQQPQQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLP        285
Q1WA39      -QQQQQPLSQVSFQQPQQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLP        284
A5JSA8      QQQQQQPLSQVSFQQPQQQYPSGQGFFQPSQQNPQAQGSFQPQQLPQFEAIRNLALQTLP        266
A5JSB5      QQQQQQPLSQVSFQQPQQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLP        269
A5JSB4      QQQQQQPLSQVSFQQPQQQYPSGQGFFQPSQQNPQAQGSFQPQQLPQFEAIRNLALQTLP        265
A5JSB3      ---QQQLSSQVSFQQPQQQYPLGQGSFRPSQQNSQAQGSVQPQQLPQFEEIRNLALQTLP        265
A5JSB2      ---QQQPSSQFSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLP        268
A5JSB0      ---QQQPSSQASFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLP        263
A5JSA5      ---QQQPSSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLP        263
A5JSB6      ---QQQPSSQFSFQQPLQQYPLGQGSFRPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLP        269
A5JSA7      ---QQQPSSQVSLQQPQQQYPSGQGFFQPSQQNPQAQGSVQPQQLPQFEEIRNLALQTLP        265
A5JSB7      --QQQQPLSQVCFQQSRQQYPSGQGSFQPSQQNPQAQGSVQPQQLPQFEEIRNLALETLP        264
A5JSA4      QQQQQQPSSQVSFQQPQQQYPSSQVSFQPSQLNPQAQGSVQPQQLPQFAEIRNLALQTLP        289
A5JSA9      HQQRQQPSSQVSFQQPQQQYPSSQVSFQPSQLNPQAQGSVQPQQLPQFAEIRNLALQTLP        288
A5JSB8      HQQRQQPSSQVSFQQPQQQYPSSQVSFQPSQLNPQAQGSVQPQQPPQFAEIRNLALQTLP        284
               ::*  ** .:**  **** .*  *:*** * *****.**** ***  ******:***
```

Fig 4.4.a. Multiple sequence alignment of α- gliadin protein of different gliadin gene in *Triticum aestivum.* Single fully conserved residues are denoted by asterisk (*). Conservation between groups of strongly similar properties are denoted by colon (:). Conservation between groups of weakly similar properties are denoted by period (.).

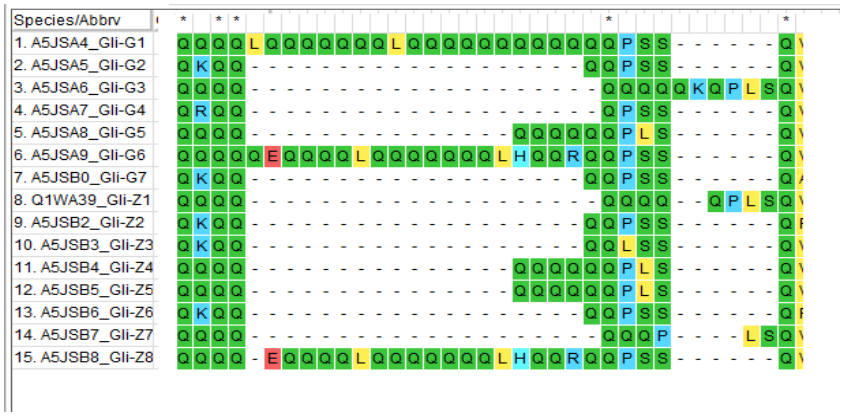

Fig 4.4.b. Highly variable region among the 15 α-gliadin proteins (Mega 7 software).

4.5. GC-MS Analysis for the secondary metabolites

The 25 wheat samples after derivatization with TMSH (trimethylsulfonium hydroxide) were submitted for GC-MS analysis for metabolomic profiling. The solvent and derivative gave about 21 peaks whereas the oldest wheat variety (C-306) showed 48 peaks for the various metabolites (Figure 4.5). The other old wheat varieties such as Chottilerma Sonalika gave the highest metabolite peaks of 63 and 62 respectively. Some new wheat varieties such as UP-2338, HUW 206, HI-617, VL-616, UP-262, and HD-2285 showed 61, 52, 50, 44, 42 and 39 metabolite peaks respectively. Lermarajo, WH-157, DBW-39 (recent variety) and HD-2329 showed 36-32 peaks for the

metabolites. Rest 11 wheat varieties (Kalyansona, HD-2009, HD-2189, VL-421, WL-711, LOK-1, PBW-343, RAJ-3765, GW-322 and PBW-502 showed very few peaks (range from 28-12) for the secondary metabolites.

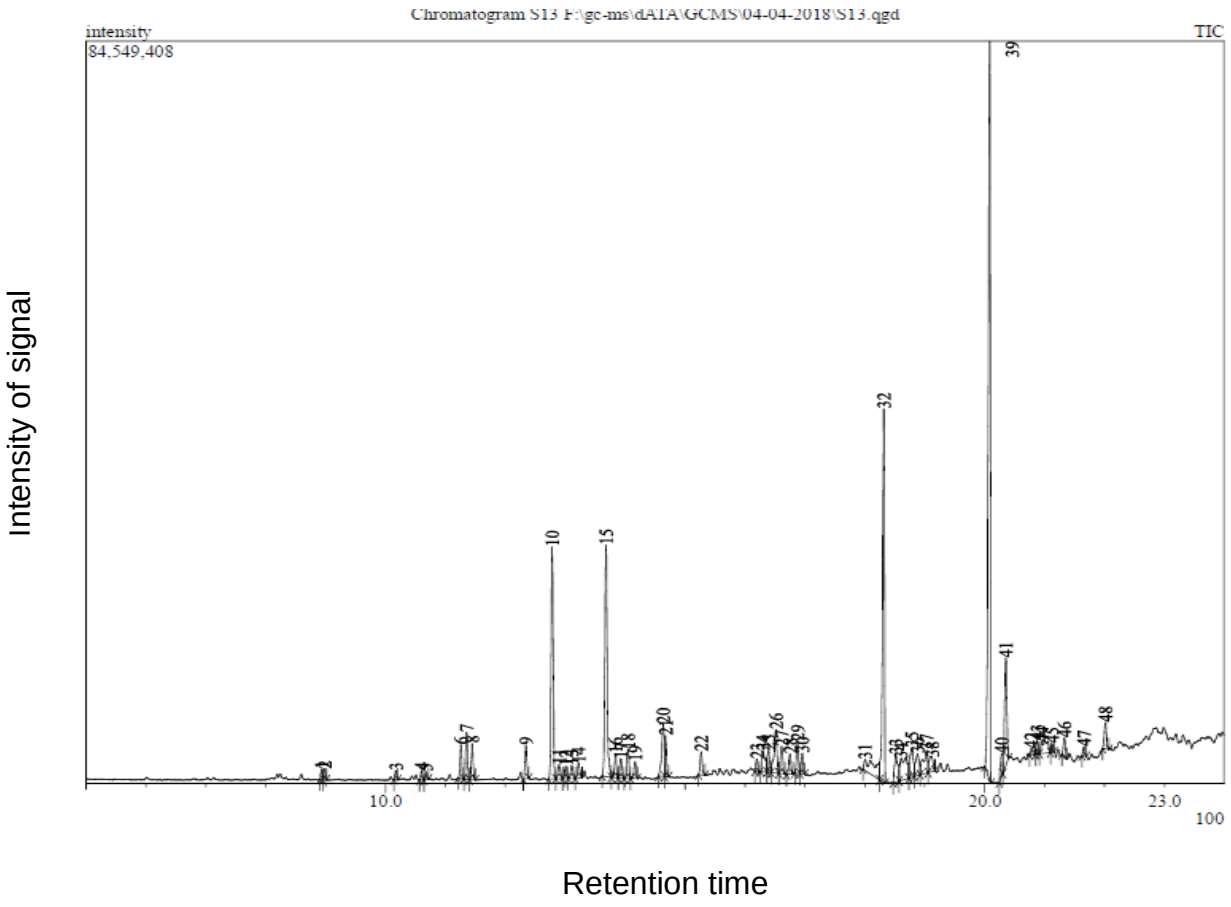

Fig.4.5. Representative Chromatogram of a wheat sample (C-306) showing the peaks for the secondary metabolites.

Variety Name	No. of new metabolites
C306	22
Chottilerma	36
Kalyansona	8
Lerma Rajo	17
Sonalika	50
HD 2009	9
HD 2189	9
UP 262	28
VL 421	6
WH 157	14
WL 711	11
HD 2285	21
HD 2329	12
HI 617	32
HS 240	9
HUW 206	36
LOK1	11
VL 616	23
HS 295	48
PBW 343	7
RAJ 3765	6
UP 2338	45
GW 322	7
PBW 502	11
DBW 39	20

Table 4.4 List of secondary metabolites present in the wheat varieties.

Metabolite profiling is key for functional annotation of genes and allowing a comprehensive understanding of cellular responses to variations in biological conditions. The key fatty acids identified in various wheat samples are tetradecanoic acid, eicosanoic acid, Pentadecanoic acid. Alkane hydrocarbon such as Dodecane, Hexadecane and Heptadecane were also identified using GC-MS analysis. Organic acids/alcohols detected from wheat were Octanol, Malonic acid, Benzoic acid, 2-hydroxyheptanoic acid, 3-hydroxybutanoic acid, 3-hydroxyoctanoic acid etc.

CHAPTER - 5

DISCUSSION

Discussion

The concentration of different proteins as estimated by Bradford method reveal that the minimum protein present in the 25 varieties is the gliadin protein. The total wheat protein concentration is nearly same in all varieties except for VL421, WH157 and WL711. The gluten protein concentration in all the varieties is near about same and are in the range of 3-4 µg/µl.

The results from SDS-PAGE analysis by densitometry of gluten protein indicate differential banding pattern for different varieties and the overall degree of variation is quite high in varieties HUW206, LOK1, VL616, HS295, PBW343, RAJ3765, PBW502, DBW39, GW322 and UP2338.Results of analysis by densitometry of the above wheat varieties are presented in table 18-27.The result recorded by comparing wheat variety C306 with the other varieties show that the total number of bands varies from 3-22. There is also difference in the intensity of the common bands present. In the above reported varieties which are from the year 1983-2010, huge variation in the total number of bands is seen. In varieties HUW206, LOK1, VL616, HS295, PBW343, RAJ3765, GW322 and UP2338.The following molecular weight bands are absent but these are present in C306: 53-41kDa and 25kDa. Also there are some extra bands present which are 20kDa and 250kDa in size. While on the other hand, 37kDa and 250kDa molecular weight bands are extra and 25kDa bands are absent, in the varieties PBW 502 and DBW39. The possible reason for this significant variation in the banding pattern could be repetitive breeding which might have caused addition/deletion of the bands in the varieties.

In the present study dendrogram was calculated from Jaccard similarity coefficient un-weighted pair group method with averages constructed by gluten bands. The dendrogram as a whole represent less heterogeneity because most of the varieties were present in the same cluster. In the previous study, SDS-PAGE gels cluster analysis of high and low molecular weight glutenin protein was conducted in 15 different varieties namely Nacozari-76, Ouqab, Tatar-96, Bakhtawar-92, Yecura-70, Raj, Bakkar, Sulilman-96, Pirsabak-05, Maria, Khyber-87, Fakhr-e-Sarhad, Pirsabak-04, Inqulab-91 and Rawal87 to check variations among varieties (Shuaib *et al.*, 2010).

Multiple sequence alignment of 15 α- gliadin protein from two different genes G and Z revealed that the maximum variation in amino acid sequences lie in the region starting from 222-259. This highly variable region contains serine, proline and glutamine amino acids. In the previous work, sequence similarities and degree of conversation at amino acid level of Gc protein was performed in order to comprehend the evolution of Gc protein from fish to mammals (Anwar *et al.,* 2013).

The GC-MD data showed very interesting results where most of the old wheat varieties showed high number of metabolite peaks as compared to the recent ones. Previously, in some studies bioactive phytochemicals such as phenolic acids, alkylresorcinols, tocols, sterols, folates, betaine, choline, dietary fibre components and polar metabolites showed only partial discrimination between the ancient and modern types of wheat (Shewry et al, 2017). Zeigler and his group (2015) has reported that composition of some of the metabolites such as alkylresorcinols can be used to discriminate between the species where as some of the phospholipids phosphatidyl choline and lysophosphatidyl choline vary with the agricultural practices such as conventional methods versus the new methods.

It is also important to mention that the genetic and environmental factors have a high impact on grain protein composition so while doing any comparative study these factors are very crucial. The extensive studies need to compare samples grown under different conditions (and on different continents) and on a wider range of genotypes of ancient and modern wheat species.

Summary and conclusion

Prolamins which primarily function as storage protein in wheat endosperm comprises of gliadin and glutenin. Consumption of gliadin protein in the diet is associated with clinical disorders like celiac disease, wheat intolerance and wheat allergy.α and γ-gliadins are majorly responsible for the allergenic reactions triggered by ingestion of wheat. In this paper a detailed study of variability of wheat and protein fractions was done.

Total wheat protein, gluten protein and gliadin protein each of 25 wheat samples were estimated. SDS-PAGE was performed for total wheat protein and gluten protein. Based on the SDS-PAGE analysis of gluten protein cluster analysis was done. Our results showed that irrespective of their origin wheat varieties showed differential banding patterns. Wheat varieties showed clustering purely on the basis of their banding pattern and not on their origin. From this work it can be concluded that evaluation of variation and identification of wheat varieties by SDS-PAGE is simple and early approach.

The GC-MS data also showed some very significant changes in the number of metabolite peaks but was not very clear to discriminate old varieties from the new ones.

Future Prospective

1. Study will be extended to analyze the gliadin genes that would be reflected in differences in prolamin proteins among wheat species and in particular between diploid and tetraploid wheat.
2. The immunotoxicity of the gliadin and gluten protein will be tested on various human colon cell lines.

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Triticum spp. having different degrees of ploidy. *Journal of Cereal Science*, **65**, 244-251.

URKUND

Urkund Analysis Result

Analysed Document:	Aarti for urkund_May 2018.docx (D38985703)
Submitted:	5/22/2018 8:17:00 AM
Submitted By:	monisha.dhiman@gmail.com
Significance:	5 %

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3-Final Thesis-Anuradha.docx (D15293429)
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