Development of protein analysis method and its application to the improvement of wheat seed quality

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Contents

List of Abbreviations

PAGE	polyacrylamide gel electrophoresis
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
LC	liquid chromatography
2-DE	two-dimensional gel electrophoresis
IPG	immobilized pH gradient
IEF	isoelectric focusing
CBB	coomassie brilliant blue
ESI	electrospray Ionization
MALDI	matrix-assisted laser desorption ionization
TOF	time of flight
HPLC	high-performance liquid chromatography
RP-HPLC	reversed-phase high-performance liquid chromatography
PMF	peptide mass fingerprinting
PVDF	polyvinylidene fluoride
EST	expressed sequence tag
ICAT	isotope-coded affinity tag
MS	mass spectrometry
MS/MS	tandem mass spectrometry
EST	expressed sequence tag
PCR	polymerase chain reaction
HMW-GS	high-molecular-weight glutenin subunits
LMW-GS	low-molecular-weight glutenin subunits

General introduction

In Korea, challenges in wheat breeding for seed quality, when compared to the United States, encompass issues such as pricing, diseases, yield stability, field supervision, gene mutations, bread-making quality, and palatability. To address these challenges, proteomic analysis is crucial, though its current utilization is limited. Recent studies indicate the absence of a singular optimal approach in proteome technology. Fractionation into subcellular compartments has proven beneficial in enhancing proteome profiling. A proteomic survey is underway, focusing on a straightforward sample preparation method for Cleveland peptide mapping and identifying seed embryo proteins in rice through 2-DE. In Korean breeding programs, extensive studies on gluten allelic variation have been conducted to manipulate end-use quality and enhance wheat quality.

Rice serves as a staple food for approximately half of the global population, primarily in Asia and Africa, where the population is expected to double in the next 50 years. However, increasing rice production faces growing challenges due to diseases and drought. Meeting the needs of the expanding population requires the development of new high-yielding rice varieties with elevated protein content. This is particularly crucial for the people of South and Southeast Asia, where rice dominates daily food consumption (Islam *et al.* 1996). To attain breeding objectives for high-yield and high-protein rice, innovative tools are essential to uncover the extensive information concealed within the rice proteome.

Proteomes of plant tissues, specific cell types and even subcellular compartments, such as chloroplasts, are complex with hundreds to thousands of protein products and an unknown amount of transient and stable protein-protein interactions. This complexity and dynamic nature make proteome analysis into an enormous challenge. From recent studies, it is quite clear that there is no single optimal, approach to capture all proteome information to attain the current status of proteome technology. Profiling of proteomes clearly benefits from fractionation into subcellular compartments, also because knowing the exact subcellular localization(s) of a protein is often important to determine its functional role and should be one of the objectives of proteomics. This fractionation directly coupled to MS, via 1-D or 2-D on-line chromatography (e.g. MUDPIT) and/or different off-line fractionation techniques (e.g. 1-D gels, off-line chromatography, affinity purifications, 2-DE gels.

However, if the *N*-terminal amino groups of proteins are modified, we cannot determine the *N*-terminal sequences of the proteins by Edman degradation. In fact, there are many proteins

in rice with blocking groups at the *N*-terminus. Currently, Cleveland peptide mapping, followed by Western blotting/gas-phase sequencing after separation by 2-DE is the only method available to efficiently obtain the internal sequence data (Hirano *et al*. 1992). In this method, proteins are separated by 2-DE, electroeluted, dialyzed, and lyophilized. The resultant protein samples are then subjected to Cleveland peptide mapping (Cleveland *et al*. 1977). However, this method is time-consuming; it takes an especially long time to perform the electroelution, dialysis, and lyophilization. Here, we report a simple and relatively rapid sample preparation method for Cleveland peptide mapping and its applications identify the seed embryo proteins separated by 2-DE in rice proteome analysis.

Recently, the identification of predicted gene products at the protein level bridges the gap between genome sequencing data and protein function, and it referred to as functional genomics (Pandey and Mann, 2000). In this respect, the techniques of the linking of proteins to EST- databases techniques is a powerful strategy for the initial identification of previously unknown protein components and for their assignment to particular EST information. Our work demonstrated the power of the rice genomics and proteomics approach. The large percentage of new non-characterized proteins detected in our present study underlines the need for further efforts in the investigation of rice proteins. We report the linking of embryo proteins and EST-databases for functional analysis of rice embryo proteins. In the preparation of protein samples for proteomic analyses of rice, the general sample preparation procedure, involving lysis buffer extraction (O'Farrell, 1975) and a subsequent TCA precipitation (Ramani and Apte, 1997), is the current method of choice. This method has been utilized in the past to extract proteins from rice tissues, including the leaves, roots, and stems. However, this method has proven somewhat ineffective for the detection of rice seed proteins, as the predominant glutelin and glutelin-like proteins tend to persist even after sample preparation. Thus, an alternative preparation method is required, which efficiently removes glutelins and effectively extracts non-glutelin minor proteins.

To achieve the breeding goals for high yield and high protein wheat, innovative tools are essential to identify the huge information hidden in the wheat proteome, which controls all of the characteristics of wheat plants. So, we have a great deal of information of about DNA and are only now entering the age of genomics the age of understanding the assembled genetic information derived from the genome. The genomics revolution has changed the paradigm for

the complehensive analysis of biological processes and systems. It is now hypothesized that biological processes and systems can gene expression patterns from cells or tissues representing different states. To test this hypothesis, it is essential that methods for the precise measurement of gene expression be developed and applied. Several methods, including serial analysis of gene expression, oligonucleotide and cDNA microarrays, and large-scale sequencing of expressed sequence tags have been developed to globally and quantitatively measure gene expression at the mRNA level (Lashkari *et al.* 1997; Velculescu *et al.* 1995). The discovery of post-transcriptional mechanisms that control rate of synthesis and half-life of proteins (varshavsky, A. 1996) and the ensuing nonpredictive correlation between mRNA and protein levels expressed by a particular gene (Futcher et al. 1999; Gygi *et al.* 1999) indicate that direct measurement of protein expression also is essential for the analysis of biological processes and systems.

Glutelin family proteins (hereafter referred to glutelins) are employed as a nitrogen source during seed germination. The glutelins constitute the major rice seed storage proteins, comprising approximately 70% of total rice proteins (Takaiwa *et al.* 1991). Glutelins, due to their presence as the predominant protein, tend to hinder the detection of less abundant non-glutelin proteins. Rice seed proteins obtained via the protein precipitation method, using lysis buffer and TCA, were found to be predominantly glutelins, according to the results of SDS-PAGE and MS analysis. Therefore, a new sample preparation method is required for the removal of glutelins, allowing the detection of minor proteins that were previously hidden by the preponderance of glutelins. Masking effects by abundant proteins have also likely been observed in proteomic analyses of higher eukaryotic organisms.

Gluten protein composition determines the rheological characteristics (strength and extensibility) of flour dough and is the key constituent responsible for differences in end-use suitability (Butow *et al.* 2003). The gluten proteins consist of the monomeric gliadins and polymeric glutenins, where glutenins contain high and low molecular weight glutenin subunits. Wheat grain research has focused on the detailed analysis of gluten proteins to better understand those aspects of protein composition accounting for the unique properties of flour (Skylas *et al.* 2005). Variations in the types of glutenin subunits correlate with quality variations among wheat cultivars, probably by affecting the molecular weight distribution of glutenin polymers (Gupta *et al.* 1993; Gupta and Shephard 1990a, b). Although the non-gluten

protein classes such as albumin and globulin occupy a smaller percentage of endosperm protein, these proteins play critical roles in cellular metabolism, development, and responses to environment.

Wheat is the staple food of millions of people. It is also an important part of the daily diet of many millions more. Only rice challenges wheat for the title of most important food grain in the world. Approximately two-thirds of the wheat produced in the world is used for human food and about one-sixth is used for livestock feed. Industrial uses, seed requirements, and post-harvest losses account for the remaining withdrawals from the world wheat granaries. Market opportunities are influenced by a country's stage of economic development. Therefore, wheat consumption should not be looked upon as a constant for any particular region or country. As consumer income increases wheat is gradually replaced by more expensive foods, particularly meat. As a result, wheat consumption in more affluent countries often depends upon its use for both human food and livestock feed.

Compared with other major cereals like rice and maize, wheat has unique end-use traits that are important for making a variety of globally consumed foods, such as various types of bread and noodles (Rasheed *et al*. 2014). The glutenins can be further divided into two subfamilies, high molecular weight glutenin subunits (HMW-GSs) and low molecular weight glutenin subunits (LMW-GSs), while gliadins contain four subfamilies, α/β-, γ-, δ- and ω-gliadins (Veraverbeke *et al.* 2002; Anderson *et al*. 2012; Wan *et al*. 2013). Thus, variations in the relative amount and composition of glutenins and gliadins have important effects on gluten functionality, dough viscoelasticity, and end-use quality (Delcour et al., 2012). LMW-GS have not been studied so intensively as HMW-GS due to their complex banding patterns observed and the mobility which overlaps with gliadins (Gupta and Shepherd 1990; Jackson *et al*. 1996). For these reasons, the study on the function of LMW-GS and the effect of their allelic variations on the quality of wheat has received far less attention than that of HMW-GS.

Grain hardness is one of the most important quality characteristics of wheat (*Triticum aestivum* L.). It is a significant property of wheat grains and relates to milling quality and end product quality. Grain hardness is caused by the presence of puroindoline genes (*Pina* and *Pinb*). Wheat hardness is affected especially by genetic factors (Turnbull *et al*. 2002). Starch granules of different sizes are coated by a protein matrix created predominantly by gluten proteins. Differences in wheat hardness are due to the adhesion of storage proteins to starch

granules (Faměra *et al*. 2004). This study was conducted to elucidate the relationships among puroindoline genotypes, grain properties and milling performance, as well as to determine the influence of puroindoline genotypes on physico-chemical properties of flour and qualities of bread, white salted noodles, and sugar-snap cookies made from Korean wheat cultivars in order to provide useful information for improving wheat quality in Korean wheat breeding programs.

In our proteomic investigation, we carried out an analysis focused on applying protein analysis technologies to enhance the quality of crop seeds. This involved the development of a straightforward and relatively rapid sample preparation method for Cleveland peptide mapping. The applications of this method were then explored to identify seed embryo proteins through 2-dimensional electrophoresis (2-DE) in rice proteome analysis.

Furthermore, there has been extensive research over a period on the allelic variation in gluten. The objective of this research was to understand the structures and properties of gluten variations. This information serves as a foundation for manipulating and improving end-use quality, offering valuable insights for enhancing wheat quality in Korean wheat breeding programs.

Chapter-1

Development of effective Cleveland peptide mapping method

Abstract

The study initially identified 31 unblocked proteins in rice embryos using direct *N*-terminal analysis. To address the remaining 69 blocked proteins, a rapid and efficient method was developed, revealing peptide maps for 20 proteins daily. This method facilitated the determination of internal sequences for all 69 blocked proteins in rice. Among the 100 analyzed rice proteins, 28 displayed sequence similarity to proteins with known functions in SWISS-PROT and NCBI databases. Peptide mass fingerprinting (MALDITOF-MS) was employed for rice proteins separated by two-dimensional electrophoresis (2-DE). While this high-throughput method faced limitations due to incomplete rice databases and post-translational modifications, the improved peptide mapping method proved valuable for rice proteome analysis, particularly for blocked proteins. In the case of wheat, a proteomic approach was utilized for analyzing and characterizing wheat lemma proteins from the cultivar Norin 61. The process involved the extraction, solubilization, and two-dimensional electrophoresis separation of about 500 polypeptides. Separation in the first dimension utilized isoelectric focusing across two pH ranges (3.5-10.0 and 5.0-8.0). Proteins were characterized using N-terminal Edman degradation microsequencing, and sequences were submitted to NCBI and SWISS-PROT databases via the FASTA algorithm. Of the 70 submitted proteins, 39 (56%) were identified from database matches, while 8 (11%) showed no homology, and 23 (33%) did not yield N-terminal sequence data. Among the 47 wheat proteins, six had homologous sequences in the wheat genome database, but the majority lacked homologous proteins. Additionally, 33 proteins had putative homologues from other plants. Forty-four protein spots were matched to wheat ESTs out of the 47 proteins sequenced, aligning with the high percentage (89%) of total wheat cDNA deposited in the database.

Comparisons of protein expression between wheat lemma and leaf revealed fewer *N*-terminal blocks in wheat compared to rice. The study reported, for the first time, the construction of proteomes from wheat lemma at the anthesis stage. Peptide sequence analysis of seventy larger spots suggested less *N*-terminal blocking in wheat proteins. The internal sequences of eight blocked proteins were analyzed using the Cleveland peptide mapping method, with 41 out of 56 amino acid sequences corresponding to expressed sequence tags (ESTs). The expression profile of lemma proteins resembled that of leaves, with the majority of identified proteins associated with cellular metabolism. Notably, the analysis of a protein

spot present in lemma but not in leaf provided insights into future examinations of the effects of environmental stress conditions on protein expression.

1.1. Introduction

Rice, a crucial staple for half the world's population, faces challenges due to disease and drought, especially in growing populations of Asia and Africa. Meeting future demands requires developing high-yielding rice cultivars with elevated protein content. This is crucial for regions like South and Southeast Asia where rice dominates daily food consumption (Islam et al. 1996). Innovative tools are essential for exploring the vast information within the rice proteome to achieve breeding goals for high-yield and high-protein rice.

Proteome analysis involves (i) separation of proteins using two-dimensional electrophoresis (2-DE), (ii) identification of proteins and corresponding genes using peptide mass fingerprints and mass spectrometry, (iii) characterization of proteins without known function, considering aspects like amount, localization, structure, post-translational modification, enzyme activity, and protein-protein interaction, and (iv) determining the functions of proteins and genes based on data from both analyses.

In the initial step of proteome analysis, peptide mass fingerprinting with MALDI-TOF-MS is commonly used (Pappin *et al.* 1993), but it may not effectively identify proteins and genes in plants and other eukaryotes due to post-translational modifications. For eukaryotic proteins, at least a 5–6 residue peptide amino acid sequence is required (Hirano 1997). Electrospray ionization-MS is sensitive and accurate for determining partial amino acid sequences but lacks high-throughput capability (Wilm *et al.* 1996). Gas-phase protein sequencing, though not rapid or sensitive compared to MS, remains useful for determining partial amino acid sequences due to simplicity and widespread availability. Currently, a pmol amount of proteins separated by 2-DE and electroblotted onto PVDF membrane can be sequenced using a gas-phase sequencer.

Determining *N*-terminal sequences through Edman degradation is hindered by modifications. In rice, many proteins have blocked *N*-terminus. Currently, Cleveland peptide mapping with Western blotting/gas-phase sequencing after 2-DE separation is the efficient method for obtaining internal sequence data (Hirano *et al.* 1992). This process involves 2-DE separation, electroelution, dialysis, and lyophilization, but it is time-consuming. A reported method offers

a simpler and faster sample preparation for Cleveland peptide mapping (Cleveland *et al.* 1977), particularly useful in identifying seed embryo proteins in rice proteome analysis.

Wheat, a staple for 90% of the global population, is primarily harvested and consumed in North America, Australia, and Europe. Population growth in these regions is anticipated to double in the next 50 years. However, escalating wheat production faces challenges from environmental stresses like disease, drought, and cold. Developing resilient wheat plants is crucial. Innovative tools are essential for identifying hidden genetic information in wheat DNA, governing plant characteristics, to advance wheat breeding.

Since the initiation of the genome sequencing project a decade ago, genomic information has been growing exponentially. Complete genome sequence data is now available for many organisms, including higher animals like humans and plants such as rice and Arabidopsis. These resources offer valuable genetic information, such as nucleotide sequences, genetic maps, and DNA markers for wheat. However, this information alone cannot address the underlying issues in complex biological systems. Some of these problems can only be addressed by examining the network of gene products (Islam *et al.* 2002; 2003). This has led to the conclusion that there is no strict linear relationship between genes and the protein complement of a cell (Pandy and Mann 2000). Therefore, analyzing the proteome is essential to fully utilize genomic information.

Recent advances in proteomic techniques, including two-dimensional electrophoresis, image analysis, microsequencing, and mass spectrometry, have provided a unique opportunity to examine the protein complement of the whole genome quickly. In these techniques, proteins are primarily separated by 2-DE, compared by image analyzers, and then identified based on peptide-mass fingerprints and partial amino acid sequence databases. Proteins of unknown function are characterized in terms of amount, localization, structure, post-translational modification, enzyme activity, and protein-protein interaction. The functions of various proteins and genes are determined using data obtained from both protein and gene analyses. The combination of these techniques has facilitated large-scale proteome analysis in plants, addressing aspects like the control by structural genes/regulators in physiochemical properties of proteins (Thiellement et al. 1986; Colas des *et al.* 1985), characterization of protein composition in developing grains (Skylas *et al.* 2000), and classification of wheat varieties (Thiellement *et al.* 1999).

Recently, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) peptide-mass fingerprinting is commonly employed for protein and gene identification (Shevchenko *et al.* 1996). Despite its high throughput, it is not always effective for identifying proteins and genes in plants and other eukaryotes. This limitation arises because plant proteins are frequently post-translationally modified, making it challenging to identify proteins and genes through peptide-mass fingerprinting alone. To identify proteins and genes, the amino acid sequence of at least a peptide consisting of 5-6 residues is required (Hirano 1992). Mass spectrometry techniques, such as electrospray ionization quadrupole time-of-flight (ESI Q-TOF/MS), can be utilized to determine partial amino acid sequences of proteins separated by 2-DE (Wilm *et al.* 1996). While highly sensitive and accurate, this method is not high-throughput.

Innovative tools are vital for achieving breeding goals in high-yield, high-protein wheat. They help unlock hidden information in the wheat proteome, governing all plant characteristics. As we amass DNA data, we are entering the genomics era, transforming the analysis of biological processes. The hypothesis suggests gene expression patterns represent different states in processes and systems. To test this, precise methods for measuring gene expression must be developed and applied.

Several methods, such as serial analysis of gene expression, oligonucleotide and cDNA microarrays, and large-scale sequencing of expressed sequence tags, have been developed to globally and quantitatively measure gene expression at the mRNA level (Lashkari *et al.* 1997; Velculescu *et al.* 1995). The discovery of post-transcriptional mechanisms controlling the rate of synthesis and half-life of proteins (Varshavsky, A. 1996) and the nonpredictive correlation between mRNA and protein levels expressed by a particular gene (Futcher *et al.* 1999; Gygi *et al.* 1999) indicate that direct measurement of protein expression is essential for analyzing biological processes and systems.

In wheat, a significant amount of expressed sequence tag (EST) information is now available in the genome database constructed by the genome sequence project in the USA. To maximize the use of this database, a gas-phase protein sequencing method, such as Edman sequencing, has primarily been used to determine protein sequence information (Hirano 1997; Kamo *et al.* 1995; Komatsu *et al.* 1994; Woo *et al.* 2002). Using this method, only 31 N-terminally unblocked proteins out of 100 proteins resolved in two-dimensional electrophoresis (2-DE)

could be determined, and after a substantial improvement in this method, the internal sequences of all 69 blocked proteins were determined (Woo *et al.* 2002).

This study aimed to create 2-DE reference maps of expressed proteins in developing wheat lemma and leaf, utilizing *N*-terminal microsequencing for protein identification. The obtained information reveals the complex heterogeneity of proteins in wheat lemma and leaf, offering a foundation for future research on genotypic and environmental interactions. This study underscores how understanding the proteome can complement gene-level studies.

1.2. Materials and methods

1.2.1. Plant materials and sample preparation

Rice (*Oryza sativa* L.) (Japonica cv. Nipponbare) seed embryos were used in this experiment. Molecular marker proteins were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Wheat plants (*Triticum aestivum* L. cv. Norin 61) were grown in the RIKEN phytotron, Wako under a day/night temperature regime of 24/18℃ during growth until maturity. Lemma, palea and leaf samples were collected at 1-day pre-anthesis and 1-day post-anthesis (DPA) during development lemma, palea and leaf were used for this analysis. Lemma, palea and leaf were isolated from fresh flolet, freeze-dried and then ground to obtain sample. Molecular marker proteins were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden).

1.2.2. Two-dimensional gel electrophoresis

In rice, a portion (100 mg, protein content ~10 mg) of embryos were homogenized with 1 mL of lysis buffer (O'Farrell 1975) and centrifuged at 15000×*g* for 5 min. The resultant supernatant (50 µL) was subjected to 2-DE (Hirano 1982). We applied the sample solution to the acidic side of the isoelectric focusing gels for the first dimension, and anodic and cathodic electrode solutions were filled in the upper and lower electrode chambers, respectively (Ito *et al*. 2000). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension was performed with 15% separation and 5% stacking gels. The isoelectric point and relative molecular weight of each protein were determined using molecular weight marker proteins (Amersham Pharmacia Biotech). After staining, the electrophoresis patterns

were scanned using a flatbed scanner, and the data were analyzed using PDQuest software Version 6.1 (Bio-Rad Laboratories, Hercules, CA, USA). I wheat, a portion (250 mg, protein content~10 mg) of lemma, palea and leaf were homogenized with 0.5 mL of lysis buffer (O`Farrell, 1975) and centrifuged at 15000×g for 5 min. The resultant supernatant (100 ul) was subjected to 2-DE (Woo *et al.* 2002). We applied the sample solution to the acidic side of the isoelectric focusing gels for the first dimension, and anodic and cathodic electrode solutions were filled in the upper and lower electrode chambers, respectively (Ito *et al.* 2000). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension was performed with 17 % separation and 5% stacking gels. Protein spots in 2-D gels were visualized by Coomassie Brilliant Blue (CBB) R250 staining. The isoelectric point and relative molecular weight of each protein were determined using molecular weight marker proteins (Amersham pharmacia Biotech). After staining, the electrophoresis patterns were scanned using a flatbed scanner, and the data were analyzed using PDQest software Version 6.1 (Bio-Rad Laboratory, Hercules, CA, USA).

1.2.3. *N*-Terminal sequence analysis

In rice, following separation by 2-DE, proteins were electroblotted onto a PVDF membrane (ProBlot; Applied Biosystems, Foster City, CA, USA) and detected by Coomassie Brilliant Blue R-250 staining (Hirano and Watanabe 1990). The blots were cut out of PVDF membranes and applied to the upper glass block of the reaction chamber in a gas-phase protein sequencer (491; Applied Biosystems). Edman degradation was performed according to the standard program supplied by Applied Biosystems. The released phenylthiohydantoin amino acids were identified by the on-line system of high-performance liquid chromatography (120A; Applied Biosystems). In wheat, following separation by 2-DE, proteins were electroblotted onto a PVDF membrane (ProBlot, Appplied Biosystems, Foster City) and detected by Coomassie Brilliant Blue R250 staining (Hirano *et al.* 1990). They were cut out of PVDF membranes and applied to the upper glass block of the reaction chamber in a gas-phase protein sequencer (491, Applied Biosystems). Edman degradation was performed according to the standard program supplied by Applied Biosystems. The released phenylthiohydantoin amino acids were identified by the on-line system of high performance liquid chromatography (120A, Applied Biosystems).

1.2.4. Internal amino acid and sequence analysis and identification of rice and wheat

In rice, proteins were separated by 2-DE and stained with Coomassie Brilliant Blue R-250. After destaining, the gel was sandwiched between filter paper (Whatman 3 mm) and polyethylene film, and dried with gel drier (Bio-Rad Laboratories). After drying, the gel with the filter paper was removed, and gel pieces containing protein spots were excised with a cutter. The gel pieces were chopped into smaller pieces and inserted in the sample well of the stacking gel for SDS-PAGE. One hundred _L of the electrode solution was added to the dried gel pieces. After incubation for 1 h, 20 _L of 2_diluted SDS sample buffer containing 10 _L of *Staphylococcus aureus* V8 protease (Pierce, Rockford, IL, USA) (0.1 _g/mL) in deionized water was overlaid on the sample solution. Electrophoresis was performed until the sample and protease were stacked in the upper gel and interrupted for 1 h to digest the protein (Cleveland et al. 1977). Electrophoresis was then continued, and the separated digests were electroblotted on the PVDF membrane and subjected to gas-phase sequencing (Hirano and Watanabe 1990). The amino acid sequences obtained were compared with those of proteins compiled in the protein sequence databases (SWISS-PROT and NCBI) using the FASTA or BLAST algorithms. The cDNA or EST sequences obtained in the Rice Genome Research Project have been translated into protein sequences and compiled as a database. Using this database, the cDNAs encoding the proteins partially sequenced in the present study were screened according to the method described by Pearson and Lipman (Pearson and Lipman 1988). In wheat, proteins were separated by 2-DE and stained with Coomassie Brilliant Blue R250. After destaining, the gels were sandwiched between filter paper (Whatman 3 MM) and polyethylene film, and dried with gel drier (Bio-Rad Laboratories). After drying, the gel with the filter paper was removed, and gel pieces containing protein spots were excised with a cutter. The gel pieces were chopped into smaller pieces and inserted in the sample well of the stacking gel for SDS-PAGE. One hundred uL of the electrode solution was added to the dried gel pieces. After incubation for 1 h, 20 uL of 2X diluted SDS sample buffer containing 10 μL of *Staphylococcus aureus* V8 protease (Pierce, Rockford, IL, USA) (0.1 μg/mL) in deionized water was overlaid on the sample soultion (Woo *et al.* 2002). Electrophoresis was performed until the sample and protease were stacked in the upper gel, and interrupted for 1 h to digest the protein (Cleveland *et al.* 1977). Electrophoresis was then continued, and the separated digests were electroblotted on the PVDF membrane and subjected to gas-phase sequencing (Hirano and Watanabe 1990).

1.2.5. Peptide mass fingerprinting of rice

The improved Cleveland peptide mapping/sequencing was compared in efficiency of identification of proteins to the peptide mass fingerprinting by MALDI-TOF-MS (Tof-Spec II; Micromass, Manchester, UK). In MALDI-TOF-MS analysis, proteins separated by 2-DE were digested in gels according to the method described by Hellman *et al*. (1995). The digests were desalted with ZipTip (Millipore, Bedford, MA, USA) and subjected to analysis by MALDITOF-MS. Based on the peptide mass fingerprints obtained, the proteins homologous to each protein that was compiled in the SWISS-PROT database were searched using ProteinLynx software (Micromass).

1.2.6. Reversed-phase high-pressure liquid chromatography (RP-HPLC) of the Cleavage fragments of wheat

Enzymatic cleavage fragments were separated on a narrow-bore (2.1-mm id.) reverse-phase HPLC system using an Agilent 1100 series LC system. The system was equipped with a Rheodyne model 7125 sample injector with a 50ul loop. The analytical column used was a Mightysil RP-18, 2.1 X 100 mm (Kanto Chemicals). The following buffer system was used. Buffer A: 0.085% trifluoroacetic acid (Sequenal grade, Merck) in water. Buffer B: 0.075% trifluoroacetic acid in acetonitrile/H_2O, 80:20 (vol/vol). All experiments were carried out with columns at ambient temperature, at a flow rate of 100 μl/min. Peptides were detected by simultaneous monitoring at 215 nm, 254 nm, and 275 nm with a Agilient 1100 Serise Diode array detector. Tryptic digest of the sample was acidified with 2 μl of 70 % formic acid, mixed quickly in a vortex, and centrifuged for 1 min in a Microfuge at high speed. Peptide-containing fractions were collected manually into tube plate based on the UV absorption at 215 nm.

1.2.7. Homology search of amino acid sequence of wheat

The amino acid sequences obtained were compared with those of proteins compiled in the protein sequence database (SWISS-PROT and NCBI) using FASTA (http://www2.ebi.ac.uk/fasta3), or BLAST (http://www.nci.nlm.nih.gov/blast) programs.

1.2.8. Identification of cDNA encoding the sequenced protein of wheat

The both cDNAs or ESTs sequences obtained in the Wheat Genome Research Project and

NCBI (http://www/ncbi.nlm.nih.gov) have been translated into protein sequences and compiled as a database. Using this database, the cDNAs encoding the proteins partially sequenced in the present study were screened according to the method described by Pearson and Lipman (1988).

1.3. Results and discussion

1.3.1. Sample preparation for Cleveland peptide mapping

In the previous study (Hirano 1997), we separated a total of 278 proteins from endosperms and embryos of seeds, leaves, and roots on 2-DE gels and electroblotted them onto PVDF membranes. The *N*-terminal amino acid sequences of 56 proteins (~20%) from 278 on PVDF membranes were analyzed by Edman degradation. In the present analysis, the *N*-terminal sequences of 31 proteins from 100 were determined by direct *N*-terminal sequence analysis. However, the *N*-terminal sequences of the other proteins (69%) could not be determined even though sequenceable amounts (>20 pmol) were obtained; *N*-terminal blockage was considered to be the reason for this. The ratio of the putative blocked proteins was roughly comparable to that described in rice proteins by our group (Komatsu et al. 1993) and another group (Tsugita et al. 1994). To obtain the sequence information of the blocked proteins, the internal sequences of 54 proteins were determined by conventional Cleveland peptide mapping, followed by blotting/sequencing. However, it was time-consuming to obtain sequence information by this method, in which, prior to the peptide mapping, we had to electroelute the proteins from the 2-DE gels for a few hours, dialyze for a few days, and lyophilize for a day. Only then, could we use the lyophilized proteins for Cleveland mapping.

In this study, we tried to improve the sample preparation method for Cleveland peptide mapping. The proteins were separated by 2-DE and stained with Coomassie Brilliant Blue R-250. After destaining, the gels were completely dried and gel pieces containing protein spots were removed with a cutter. We directly inserted the dried gel pieces into the sample well of the stacking gel for SDSPAGE. The electrode solution for SDS-PAGE was loaded to the dried gel pieces to be swollen and equilibrated against the alkaline electrode solution. Drying of the gel was important to evaporate acetic acid contained in the Coomassie-stained gel and to increase the peptide recovery. Without drying, it was very difficult to efficiently elute proteins

from the gel pieces during SDS-PAGE. After equilibration with the alkaline electrode solution for 1 h, *S. aureus* V8 protease solution was overlaid on the sample solution and then electrophoresis was carried out. During electrophoresis, the protein was digested, and the resultant peptides were separated. The separated digests were electroblotted on the PVDF membrane and subjected to gas-phase sequencing.

Using this method, the peptide mapping of the three standard proteins, bovine α-lactalbumin, soybean Kunitz trypsin inhibitor, and bovine carbonic anhydrase, was also performed (Fig. 1). We applied 100 pmol of the proteins to the SDS-PAGE gels and recovered 7.3-13.5 pmol as initial yields of the peptides in gas-phase sequencing (Table 1). This indicates that, since the amino acid sequence of 1 pmol of protein can be determined by the gas-phase sequence if at least 14 pmol of protein is available, then we can determine the sequence. This method is not only sensitive, but also efficient. We are able to perform peptide mapping of 20 proteins per day. Therefore, the improved method can be used to efficiently prepare samples for the determination of the internal amino acid sequences of the proteins. In the present study, this method was applied to identify a number of the seed embryo proteins in the rice proteome analysis as described later.

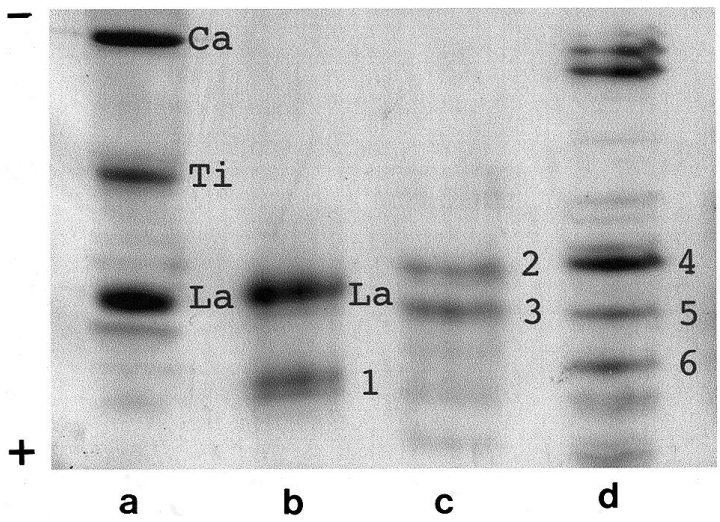

Fig. 1. Peptide map of the *S. aureus* V8 protease digests of three proteins. Low-molecular-weight standard marker proteins (100 pmol) were separated by SDS-PAGE and stained with Coomassie blue. After destaining, the gel was completely dried and then the gel pieces containing proteins were chopped into smaller pieces and inserted into the sample well for Cleveland peptide mapping. The resultant peptides on the SDS-PAGE gel were electroblotted on the PVDF membrane and subjected to gas-phase sequencing. La, α-lactalbumin; Ti, trypsin inhibitor; Ca, carbonic anhydrase; a, intact proteins; b, digests of La; c, digests of Ti; d, digests of Ca.

Table 1. Overall yields of the *S. aureus* V8 peptides obtained by Cleveland peptide mapping

Protein	Mr	Amount applied to SDS-PAGE (pmol)	Peptide obtained by Cleveland mapping			N-Terminal sequence of peptide
			Band No.	Predicted M_r	Initial yield (%)	
α-Lactalbumin	14400	100	1	8700	10.3	Y^{50}GLFQINNKIW
Trypsin inhibitor	20100	100	2	14800	7.3	G^{70}HPLSLKFDSF
Carbonic anhydrase	30000		3	12600	13.4	L^{50}DKGIGTIISP
		100	4	15800	-	Blocked (N-terminal peptide)
			5	12750	13.5	L^{117}HLVHWN
			6	9400	10.2	L^{117}HLVHWNTK

See Fig. 1 for peptide band numbers.

Previously, we reported a method for deblocking *N*-terminally modified proteins (Hirano et al. 1993). In this method, proteins were chemically or enzymatically deblocked with *N*-formyl and *N*-acetyl groups, and proteins with *N*-terminal pyroglutamic acid were transferred onto PVDF membranes. However, deblocking the protein in this method is time-consuming and not always successful, and deblocking methods for the other blocking groups are not available. The improved Cleveland peptide mapping method achieved in this study was found very useful to obtain sequence information from the blocked proteins within a very short time.

1.3.2. Application of the improved method to the identification of rice embryo proteins

Rice embryo proteins were separated by 2-DE and a total of 700 proteins were detected on the gels by Coomassie Blue staining (Fig. 2). Most embryo proteins had an isoeletric point between 5.0-7.0. Using silver staining for protein detection, we visualized more than 1000 spots on a single gel (data not shown). The proteins were automatically identified on 2-DE gels with PDQuest software based on relative molecular weights and isoelectric points (Table 2, Fig. 2). The embryo proteins were electroblotted from the 2-DE gels onto PVDF membranes and stained with Coomassie blue. Among them, 100 proteins were subjected to gas-phase sequencing, and the *N*-terminal sequences of 31 proteins were determined (Table 2), but the sequence information of the remaining 69 proteins could not be determined, suggesting that these proteins were *N*-terminally blocked.

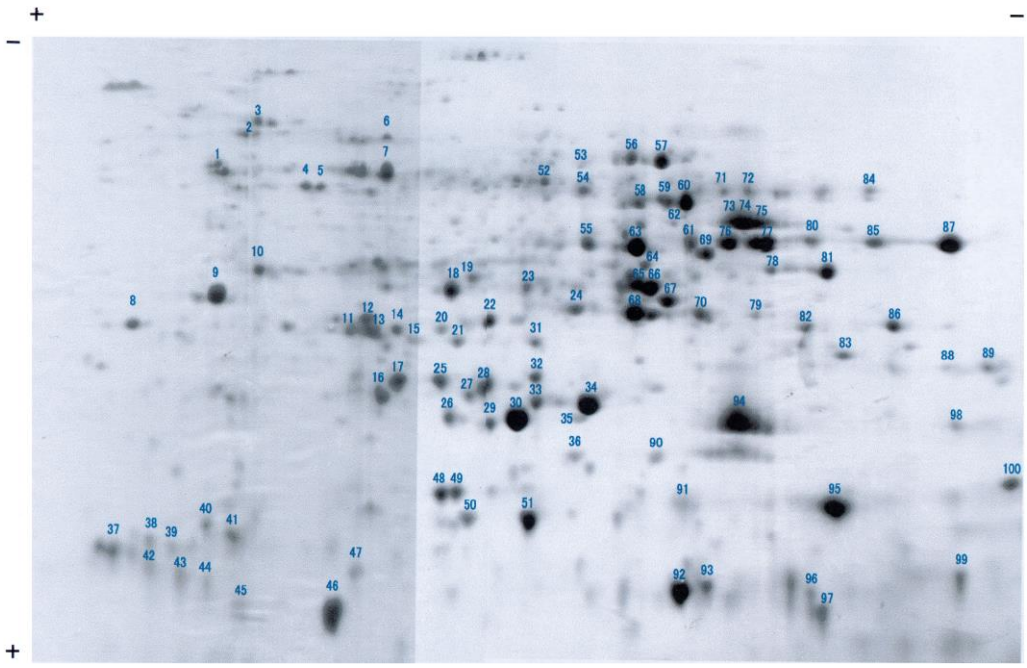

Fig. 2. Two-dimensional electrophoresis pattern of the rice embryo proteins. Proteins were separated by isoelectric focusing in the first dimension (right to left) and SDS-PAGE in the second dimension (top to bottom) and detected by Coomassie blue staining. See Table 2 for the protein identification numbers.

We attempted to determine the internal amino acid sequences of 100 proteins including these putative *N*-terminally blocked proteins by the improved Cleveland peptide mapping method. We efficiently obtained the peptide maps of 100 proteins separated by 2-DE in two weeks and a major peptide of each protein was sequenced (Table 2). We also determined the internal amino acid sequences of 76 proteins including 69 *N*-terminally blocked proteins.

Table 2. Rice embryo proteins identified on 2-DE gels

Spot No.	*N*-Terminal sequence	Internal sequence	Homologous protein	Species	Accession No.	p^I	M_r (kDa)
1	Blocked	IKSDEDSTNX				4.78	56.1
2	Blocked	GNGVWAPGNP				4.88	62.8
3	Blocked	GNDVGAPTNP				4.92	67.0
4	Blocked	DRIKXAXGAX				5.11	50.8
5	Blocked	FKQAGNMNXX				5.15	50.1
6	Blocked	SDESQGPDXX				5.38	62.3
7	SGAILAGVTTXAAN	TADASDDXLG				5.39	53.5
8	Blocked	AKQAAGSDDN				5.39	53.5
9	Blocked	AKATGTDTRM				4.46	33.5
10	Blocked	GDVLW				4.81	35.1
11	ESSRGPFNILXT					5.01	36.0
12	EAAAGPFNILGQ					5.25	33.1
13	GPKFWGGNLKKLGSK					5.35	32.7
14	ESSRGPFNILXT					5.44	33.3
15	ESSRGPFNI					5.49	32.2
16	**GRIFFVGGN (8/9)**[c)]		Triosephosphate isomerase, cytosolic (27.1 kDa)	Rice	P48494[a)]	5.38	28.2
17	SGGYNNXPVK	LAAQHEEDD				5.43	29.2
18	Blocked	AIIQSYKPANIGY LEAQAQQ				5.53	35.1
19	**GLTFGDTVPNLELDS (15/15)**[c)]		RAB24 Protein (24.1 kDa)	Rice	P52573[a)]	5.57	36.0
20	ESSRGPFNI					5.55	32.1
21	Blocked	IAWXX				5.53	32.2
22	Blocked	**IXIPXKSGYYGE QQQQPGMTR (14/14)**[c)]	Globulin precursor (α-globulin) (21.0 kDa)	Rice	P29835[a)]	5.62	33.2
23	Blocked	**SLVLAGVMHG (10/10)**[c)]	Guanine nucleotide binding protein β-subunitlike protein (36.2 kDa)	Rice	P49027[a)]	5.73	38.3
24	Blocked	HQ(F)LPNNDRHX				5.84	33.8
25	Blocked	FEVKGLFLFM				5.52	29.3
26	VGPVALPNLK	KDRRADDGK				5.55	26.3
27	Blocked	**SQSQSQKFKD (10/10)**[c)]	Glutelin type I precursor (55.8 kDa)	Rice	P07729[a)]	5.57	28.2
28	Blocked	LAAQHGEDDG				5.63	28.8
29	Blocked	XTANSDPDKA				5.63	26.0
30	Blocked	ATKLALP(E)DTPT ATYVKDAAKAG KED				5.71	26.3
31	Blocked	SWPGWGQGKP				5.75	32.1
32	Blocked	QWQWGWGFGD				5.73	30.0
33	PGLFGDTV					5.81	27.8
34	**PGLTIGDIVPNLGLD (13/15)**[c)]		RAB24 Protein (24.1 kDa)	Rice	P52573[a)]	5.85	27.5
35	Blocked	TAQRTKDAAK				5.84	26.4
36	Blocked	FSGGS				5.82	23.1
37	QLEXVEX					4.41	16.2
38	Blocked	EEEEEEEEQQT				4.56	16.9
39	Blocked	EEEEEEETPRFHP PA				4.65	16.3
40	Blocked	VAYGTKPDGGEL T				4.77	18.3
41	Blocked	EEEEEEETTT				4.88	17.5
42	EEEEEEEEEQ					4.56	15.6
43	EEEEEEEEEQ					4.68	15.2
44	EEEEEEEEEQ					4.78	14.9

Continued

Spot No.	*N*-Terminal sequence	Internal sequence	Homologous protein	Species	Accession No.	p^I	M_r (kDa)
45	Blocked	GHADMLMGGA				4.88	13.8
46	Blocked	**LDRMAREGQT (10/10)[c)]**	Embryonic abundant protein 1. (10.2 kDa)	Rice	P46520[a)]	5.26	13.0
47	Blocked	SQSQSRGWQG				5.31	15.7
48	SFVAVTVYRITTFY					5.52	20.0
49	Blocked	GDGWGGDGWG				5.56	20.0
		TAEQSTDAAN				5.56	20.0
50	Blocked	TQQQQQQRKP				5.56	18.8
51	WSGGGEF	**GNVLVISGQRSK E (13/13)[c)]**	Class I heat shock protein (16.9 kDa)	Rice	P27777[a)]	5.71	18.7
52	Blocked	**DKYGSDIDIX (8/10)[c)]**	Glyceraldehyde 3-phosphatedehydrogenase, cytosolic (36.5 kDa)	Rice	Q42977[a)]	5.76	52.5
53	Blocked	G(K)LQADRXESG				5.84	55.7
54	Blocked	SGDVGKSFRS				5.84	49.5
55	Blocked	TADASDDKLG				5.86	40.6
56	Blocked	TQQQQQQGKP				5.94	57.2
57	Blocked	IGLEAQAQQ				6.01	55.7
58	Blocked	**SQSQSQKFKD (10/10)[c)]**	Glutelin type I precursor (55.8 kDa)	Rice	P07729[c)]	5.95	50.8
59	Blocked	TQQQQQQXLP				6.06	47.5
60	Blocked	**SMIVIPASHP (9/10)[c)]**	Globulin-like protein (51.7 kDa)	Rice	AAD10374[b)]	6.09	47.5
61	**GKIKIGING(9/9)[c)]**		Glyceraldehyde 3-phosphatedehydrogenase, cytosolic (36.5 kDa)	Rice	Q42977[a)]	6.15	42.3
62	RRGEED					6.11	40.9
63	Blocked	TADASDDKLG				5.95	40.2
64	Blocked	KEYLPDIDIVX				5.96	37.2
65	Blocked	SGDVGXXFCN				5.95	35.3
66	Blocked	GNVXGAPTNPPD EE				6.02	35.2
		SVLXTESRDV				6.02	35.2
		LIQVGGVQW				6.02	35.2
67	Blocked	SQKPNNWRQG				6.05	35.1
68	Blocked	**SQSQSQKFKD (10/10)[c)]**	Glutelin type I precursor (55.8 kDa)	Rice	P07729[a)]	5.95	34.4
69	Blocked	**DKYTSDIDIV (10/10)[c)]**	Glyceraldehyde 3-phosphatedehydrogenase, cytosolic (36.5 kDa)	Rice	Q42977[a)]	6.15	36.2
70	Blocked	**FLLAGNNNRE (10/10)[c)]**	Glutelin precursor (56.8 kDa)	Rice	P14614[a)]	6.13	34.0
71	Blocked	IFIDEDATPS				6.43	49.2
72	WSPPGLWLDEDLLR					6.70	49.1
73	Blocked	AGYRHVDTAA				6.48	46.3
74	WSPPGLWLDEDLLR	SGAKLAG				6.65	44.3
75	Blocked	**SQSQSQKFKD (10/10)[c)]**	Glutelin type I precursor (55.8 kDa)	Rice	P07729[a)]	6.91	47.0
76	EGEKAD					6.32	42.3
77	Blocked	TAEQSTDAAN				6.58	41.6
		GSLLKPNXXT (8/10)[c)]	Aldolase c-1(38.8 kDa)	Rice	Q42476[a)]	6.58	41.6
78	**GKIKIGINGFGRIG RLV(17/17)[c)]**		Glyceraldehyde 3-phosphatedehydrogenase, cytosolic (36.5 kDa)	Rice	Q42977[a)]	6.55	37.5
79	HPEAIPQ	SESETDATT				6.47	34.2
80	Blocked	SQSQSAI				6.95	41.5
81	**AKIKIGING (8/9)[c)]**	**KEYKSDVNIVSN(12/12)[c)]**	Glyceraldehyde 3-phosphatedehydrogenase, cytosolic (36.5 kDa)	Maize	Q43359[a)]	7.22	37.3

Continued

Spot No.	*N*-Terminal sequence	Internal sequence	Homologous protein	Species	Accession No.	p^I	M_r (kDa)
82	Blocked	KTTXGG				6.80	33.1
83	Blocked	TQQQQQQQIP				7.41	31.5
84	Blocked	RDRRGEGSSEE EDE				7.52	49.1
85	Blocked	ATXNXNADNI				7.51	41.2
86	Blocked	**FLLAGNNNRA (10/10)[c)]**	Glutelin precursor (56.8 kDa)	Rice	P14323[a)]	7.82	33.3
87	Blocked	AGVNLNADNK	Aldolase c-1 (38.8 kDa)	Rice	Q42476[a)]	8.41	42.0
		GSLLKPNMVT PGSE (13/14)[c)]				9.01	31.0
88	Blocked	S(P)FQGGG				9.21	30.9
89	Blocked	AKGAASDATG				6.01	23.2
90	Blocked	GASPAXAAATT A				6.01	23.3
		AMATGTDTKAP GG				6.09	19.7
91	**MERPWKXDNXKXL (10/13)[c)]**		Bowman-Birk type bran trypsin inhibitor precursor (21.6 kDa)	Rice	P07084[a)]	6.09	19.7
92	Blocked	**LDRRARDDGK (10/10)[c)]**	Late embryogenesis abundant protein (10.4 kDa)	Rice	BAA96766 [b)]	6.02	14.8
93	Blocked	**AKDVPRTAEN (10/10)[c)]**	Cyclophilin 2 (18.3 kDa)	Rice	AAA57046 [b)]	6.20	14.9
94	Blocked	**YGNPVGTGAQ (9/10)[c)]**	Water-stress inducible protein RAB21 (16.5 kDa)	Rice	P12253[a)]	6.67	26.5
95	**MERPWKXDNI(9/10)[c)]**		Bowman-Birk type bran trypsin inhibitor precursor (21.6 kDa)	Rice	P07084[a)]	7.25	19.7
96	ESRRGPFN					6.85	14.7
97	Blocked	**SQSQSXKFKD(9/10)[c)]**	Glutelin type I precursor (55.8 kDa)	Rice	P07729[a)]	7.21	13.5
98	GLLGLX					9.01	26.3
99	Blocked	DAASDATXGA				8.73	15.8
100	Blocked	**MCQGGDFTRG (10/10)[c)], AKDVPRTAEN (10/10)[c)]**	Peptidylprolyl isomerase (18.4 kDa)	Rice	1084455[b)]	9.23	21.4
	Blocked	**MCQGGDFTRG (10/10)[c)], AKDVPRTAEN (10/10[c)]**	Cyclophilin 2 (18.3 kDa)	Rice	600769[b)]	9.23	21.4

[a)] Accession No. of SWISS-PROT database

[b)] Accession No. of NCBI database

[c)] (Number of residues matched with those of protein sequence in the database/number of the residues identified by sequencing). The matched residues are highlighted in bold.

The partial amino acid sequences of 100 proteins were com pared with those of the SWISS-PROT and NCBI databases by the method of Pearson and Lipman (1988). The sequences of 28 proteins were found identical or similar to those of proteins already reported in rice; the proteins included cytosolic triosephosphate isomerase (P 48494), guanine nucleotide-binding protein β-subunitlike protein (P 49027), RAB 24 protein (two spots, P52573), embryonic abundant protein 1 (P46520), 16.9 kDa class 1 heat shock protein (P 27777), Bowman-Birk type bran trypsin inhibitor precursor (two spots, P07084), water stress inducible protein RAB 21 (P12253), aldolase c-1 (two spots, P17784), cytoplasmic fructose-bisphosphate aldolase, cytosolic glyceraldehyde-3-phosphate dehydrogenase (four spots, Q 42977), glutelin precursor or glutelin type I precursor (seven spots, P07730, P14614, P14323), globulin-like protein (ADD10374), late embryogenesis abundant protein (BAA96766), cyclophilin 2 (AAA57046, S48017), and peptidylprolyl isomerase (1084455) (Table 1). Spot 81 was a protein which showed high sequence homology with the cytosolic glyceraldehyde 3-phosphate dehydrogenase (Q43359) of maize.

Although 30% of the proteins visualized in 2-DE in our study were identified, we were not able to identify the remaining (70%) because of information lacking in the rice database, even if we sequenced several internal peptides (Table 2). These include some major proteins such as spots 5, 8, 45 and 49, which could be targeted for functional analysis in rice proteome analysis. To investigate the functions of these proteins, their localization, structure and posttranslational modification are under investigation.

A total of 150 proteins separated by 2-DE were analyzed by peptide mass fingerprinting using MALDI-TOF-MS. These proteins include most of the proteins analyzed by Cleveland mapping followed by gas-phase sequencing. However, we unambiguously identified only 46 proteins by MALDI-TOF-MS. This suggests that although peptide mass fingerprinting is a sensitive high-throughput method, we cannot always easily identify the rice proteins or genes by fingerprinting because the complete nucleotide sequence of the genome in rice is not yet available and the protein database does not have enough information for the identification of unknown proteins. In addition, plant proteins are often post-translationally modified and peptide-mass fingerprinting is not very useful to identify the modified proteins. In this situation, the improved sample preparation method for the peptide mapping achieved from our research is considered to be very useful in rice proteome analysis.

Several methods, including serial analysis of gene expression, oligonucleotide and cDNA microarrays, and large-scale sequencing of ESTs have been developed to measure gene expression at the mRNA level globally and quantitatively (Velculescu *et al.* 1995). Post-transcriptional and post-translational mechanisms often control the rate of synthesis and half-life of proteins (Varshavsky 1996). Nonpredictive correlation between mRNA and protein levels expressed by a particular gene (Futcher *et al.* 1999; Gygi *et al.* 1999) indicates that direct analysis of protein expression is essential. In order to analyze the protein expression rapidly, more efficient techniques are required to be developed in the future.

1.3.3. Two-dimensional electrophoretic separation of proteins map of wheat

A master 2-D gel electrophoresis with an isoelectric focusing range of pH 3.5-10 and a potential load of 100 µl results in a gel with more than 500 features visualized by CBB staining (Figure 3). Most lemma polypeptides had a iso-electric point between 5.0-7.0. By narrowing the pH range from 7 to 1 unit/20 cm gel and by increasing the protein load to 500ul, more than 500 spots could be visualized on a single silver-stained gel (data not shown). Also, the proteins were automatically identified on two-dimensional gels electrophoresis with PDQUEST software based on molecular weights and isoelectric points. The two-dimensional electrophoresis images were aligned and matched. Mostly, the containing Ribulosebisphosphate carboxylase proteins and proteins released, from broken organelles, in order to take into account a possible adsorption, or entrapment, of soluble proteins.

Our results showed that, assuming one translated product per gene transcript, we have been able to display about 10% of the potential genomic expression following 2-DE of wheat lemma proteins. RNA excess DNA/RNA hybridization experiments showed that there are about 20,000 and 25,000 genes expressed in the pollen of *Tradescantia* and maize (Mascarenhas 1990) and of tobacco (Koltunow 1990). However, no more than 2000 of these mRNAs are the most abundant and middle abundant classes of the total mRNAs. As shown in this paper, it is possible to display more proteins on a single gel that would be encoded by these most abundant and middle abundant mRNA classes. However, it remains a big challenge in proteomics to increase the number of less abundant, hydrophobic and basic proteins that are under-represented on 2-DE gels.

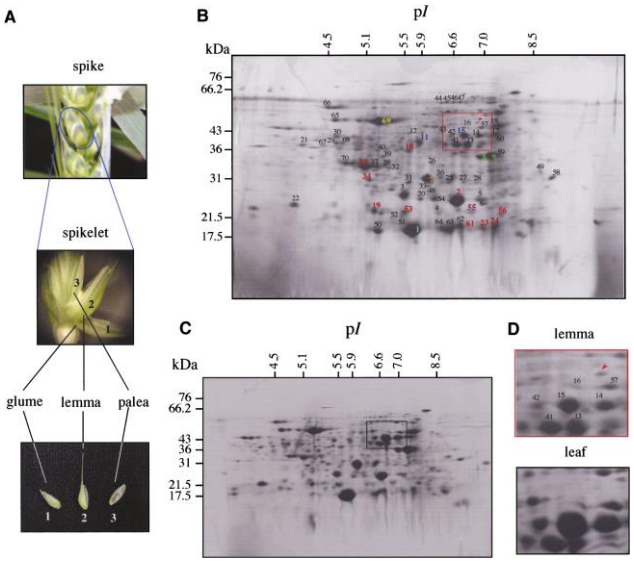

Fig. 3. Proteome analysis of wheat lemma. (A) Structure of floral tissues. A spike consists of numerous spikelets that contain glumes, lemma, and palea. (B) 2-D PAGE proteins of lemma proteins. The 2-D gel was visualized with CBB and the major spots sequenced are numbered. Numbers in the same color (except black) indicate that the protein spots correspond to the same proteins. (C). 2-D PAGE profiles of leaf profiles. (D) A magnified view of the region (upper panel; lemma, lower panel; leaf) marked by a frame in Fig. 3 B (red) and C (black).

1.3.4. Comparison of protein expression for wheat lemma and leaf

For characterization, this lemma and leaf polypeptides were subjected to *N*-terminal amino acid sequencing. In lemma specific protein, all *N*-terminal amino acid sequence was obtained: NH_2-IILFGGAGVGK-COOH and NH_2-GSEGGGGS-COOH. The comparison with sequences available in different databases showed strong similarities with two proteins.

Table 3. Wheat lemma proteins identified on 2-D PAGE

Identified protein[a)]	Spot no.	M_r	*N*-terminal/internal sequence	Position from mature protein[d)]
RuBisCo LSU (P11383)	2	25	*N*-blocked WIDTDILAAFR[b)]	31
	10	37	*N*-LSAKNYGR	178
	19	21	*N*-HIHRAMHAVID	290
	23	19	*N*-ALRMSGGDHI	315
	24	20	*N*-MSGGDHIHSGT	318
	34	30	*N*-ATAGTCEEMIKR	240
	35	34	*N*-LSAKNYGRACYE	178
	53	22	*N*-AVIDRQKNHGMH	297
	56	22	*N*-YYTPEYETKD	22
	61	19	*N*-AKALRMSGGDHIHSG	313
RuBisCo SSU (P00871)	1	19	*N*-XQVWPIEGIKKFETL	1
OEE2 (Q00434)	7	30	*N*-AYGEAANVFGKAKK	1
OEE1 (P27665)	8	36	*N*-EGAPKRLTFDEIQSK	1
	9	36	*N*-EGAPKRLTFDEIQSK	1
RcaB (AAF71272)	11	40	*N*-blocked WISGNAGEPAKL[c)]	190
	15	41	*N*-blocked WISGNAGEPAKL[c)]	190
SOD1 (AAB67990)	55	23	*N*-ATKKAVAVLKGT	48
AOS (AAO43440)	69	45	*N*-DAKSFPVLFDVA	75

[a)] See text for abbreviated names of identified proteins

[b)] Protein was digested with *Achromobacter protease* I.

[c)] Protein was digested with V8 protease

[d)] The residue numbers from the *N*-terminus of mature proteins

1.3.5. Identification of proteins spots by Edman degradation of the wheat lemma proteins

The sequence comparisons showed 47 proteins characterized in wheat with 88% to 100% identity (Table 3): plastocyanin, and glutelin precursor or glutelin type-b 2 precursor (seven spots, X05662, X14393, X15833). Sixteen proteins are newly described in buckwheat in this paper but have been described to other plant species. 40s ribosomal protein s14 (maize), ferredoxin-thioredoxin reductase catalytic chain precursor (maize, X 73549), zein-alpha precursor (22 kDa) (maize, four spots, V 01478), perredoxin-thioredoxin reductase catalytic chain precursor (maize, X 73549), peptidyl-proiyl cis-trans isomerase (maize, two spots, M55021, X68678), globulin-1 s allele precursor (glb1-s) (maize, M24845), convicilin precursor (pea, X 06398), provicilin precursor (type b) (pea), legumin b, k (fragment) (pea, three spots, X07015), nad(p)h dependent 6`-deoxychalcone synthase (soybean, X 55730), nodulin 26b (soybean, X 05092), glycine precursor (soybean, M 10962), stress-induced kin 1. Protein (arabidopsis, X51474, Z17797), putative trypsin inhibitor at2g43550 precursor (arabidopsis, AC 002335), and transcription initiation factor 11b (soybean, U31097). 50% to 100% identity in amino acid sequences was found between the peptide fragments and pyrophosphatase, Cys synthase, and aldolase. In plants, embryo proteins, a large proportion of novel or functionally uncharacterized proteins was found. The glutelin precursor protein is a member of a family of seed storage proteins characterized in various crop species and thought to be located at the organ embryo/endosperm interface. Very good probabilities were given by the FASTA or BLAST soft program for these identifications. The internal amino acid sequence of seventeen other proteins did not match any sequence in the databases.

1.3.6. ESTs in combination with protein analysis can be used for protein identification

Our results demonstrate that ESTs can be used not only for protein identification but also for protein isoform detection by protein sequence analysis. However, special care should be taken for the confidence of the matches. Matches due to missing cleavages and artificial modifications should be considered cautiously as good matches are largely free from them and poor matches contain a large number of missing cleavage and artificial modifications.

Table 4. Partial amino acid sequences of Wheat lemma proteins and their homologues found in the database

Spot No.	M_r	p^I	*N*-terminal Internal Sequence	Homologue[b)] (Organisms)	Homology[c)]	Accession No.
3	26	5.5	*N*-blocked			
			ITGDTDILAAF	RuBisCo LSU (*Lygodium lanceolatum*)	9 /10	CAC83467
4	21	6.4	*N*-blocked			
5	25	6.9	*N*-blocked			
			ITGDTDILAAF	RuBisCo LSU (*L. lanceolatum*)	9 /10	CAC83467
6	24	7	*N*-blocked			
12	41	5.6	*N*-FQAPTGDGTH	RuBisCo activase A (*Hordeum vulgare*)	10 /10	C23703
13	38	6.8	*N*-GAYDDELVKTAKTIA	Fructose bisphosphate aldolase (*Oryza sativa*)	15 /15	Q40677
14	40	6.9	*N*-GAYADELVKTAKTIA	Fructose bisphosphate aldolase (*Pisum sativum*)	14 /15	Q01516
16	43	6.8	*N*-AENIDEKRNT	RuBisCo activase A (*H. vulgare*)	10 /10	Q40073
17	41	7	*N*-AENIDEKRNT	RuBisCo activase A (*H. vulgare*)	10 /10	Q40073
18	43	7.1	*N*-blocked			
			ISGPEMEI	Hypothetical protein FLJ10858 (*Rattus norvegicus*)		
20	25	6	*N*-AGSISADRVPDMSKR	Rieske Fe-S precursor protein (*P. sativum*)	12/15	P26291
21	38	4.2	*N*-GTVAAIKVGP	Amino acid transport protein (*Lactobacillus plantarum*)	8/10	NP_784593
22	23	4.1	*N*-GTMAAWIMGP	Ammonium transporter (*Chlamydomonas reinhardtii*)	7/10	AAL85345
25	30	6.5	*N*-AKEAAPVSGD	Unknown		
26	34	6.2	*N*-blocked			
			ISGNHGEPAKL	RuBisCo activase (*Chlorococcum littorale*)	9/10	CAA71667
27	30	6.7	*N*-ARRFAVNVN	AcylCoA dehydrogenase (*Caenorhabditis elegans*)	7/9	NP_491859
28	30	6.8	*N*-blocked			
29	39	4.7	*N*-APIKIGI	GPD (*Dianthus caryophyllus*)	7/7	P34921
30	40	4.7	*N*-GKIKIGINGFGRIGR	GPD (*H. vulgare*)	15/15	P26517
31	30	5.6	*N*-blocked			
32	33	5.4	*N*-blocked			
			IELAPVVDG	Putative large ATPbinding protein (*Streptomyces coelicolor*)	7/8	NP_627578
33	26	6.1	*N*-SRVDSFSKSDI	Ribulose5phosphate3epimerase (*Arabidopsis thaliana*)	10/11	NP_200949
36	31	6.4	*N*-blocked			
37	34	5.2	*N*-SGNELPENKV	Translation initiation factor-like protein (*Plasmodium falciparum*)	7/10	NP_704285
38	34	5.3	*N*-blocked			
39	34	5.3	*N*-SYEYVSQGLKKY	RuBisCo activase A (*H. vulgare*)	11/12	Q40073

40	36	5.2	*N*-INGFGRIGVL	GPD (*Buchnera aphidicola*)	10/10	Q07234
41	39	6.7	*N*-SAYADELVKTA	Fructose 1,6 bisphosphate aldolase (*Avena sativa*)	11/11	AAF74220
42	42	6.6	*N*-blocked			
43	45	6.4	*N*-AENIDEKRNTD	RuBisCo activase A (*H. vulgare*)	11/11	Q40073
44	57	6.4	*N*-blocked			
45	57	6.5	*N*-blocked			
46	57	6.6	*N*-blocked	Adenosine diphosphate glucose pyrophosphatase (*Triticum aestivum*)	11/12	CAC85479
47	57	6.7	*N*-blocked			
48	27	6.3	*N*-LTQDFCVADLAC			
49	33	8.7	*N*-CFIAEKN	Unknown		
50	19	5.2	*N*-XQVRPIEGIKKF	RuBisCo SSU (*T. aestivum*)	10/11	CAA25058
51	21	5.5	*N*-VKAVAVLTGSE	Cu /Znsuperoxide dismutase (*Zanteaeschia aethiopica*)	11/11	O65174
52	20	5.4	*N*-VKAVAVLTGSEG	Cu /Zn superoxide dismutase (*Z. aethiopica*)	12/12	O65174
54	25	6.3	*N*-blocked			
57	43	6.9	*N*-ALAYDISDDQ	RuBisCo activase B (*T. aestivum*)	9/10	AAF71272
58	30	9.1	*N*-blocked			
59	37	7.2	*N*-LLRPTELK	Unknown		
60	41	7.4	*N*-XGPETTGVIQRM	Glutamine synthase (*H. vulgare*)	11/11	P13564
62	20	6.7	*N*-XQVRPIEGIKKF	RuBisCo SSU (*T. aestivum*)	10/11	CAA25058
63	19	6.6	*N*-AQVWPIEGIKKFETL	RuBisCo SSU (*T. aestivum*)	14/15	CAA25058
64	19	6.5	*N*-XQVRPIEGIKKF	RuBisCo SSU (*T. aestivum*)	10/11	CAA25058
65	45	4.7	*N*-SLPSSSNGGSDR	Putative SWH1 protein (*A. thaliana*)	9/12	NP_192983
66	54	4.5	*N*-blocked			
67	38	4.4	*N*-AADSKNILI	Hevein-like protein (*Eutrema wasabi*)	7/9	BAC16357
68	38	4.8	*N*-KLKVAINDGFGRI	GPD (*A. thaliana*)	12/13	JQ1286
70	33	4.8	*N*-GPAKNYGRA	RuBisCo LSU (*Tovara virginiana*)	8/9	AAN71858

[a)] Bold letters indicate the presence of corresponding wheat ESTs with 100% of homology to the regions.

[b)] See text for abbreviated names of homologous proteins.

[c)] Number of amino acid residues matched to the reference sequence/total number of amino acid residues.

Most of the identified ESTs matched to the proteins of different species indicating either the nonexistence of these protein isoforms in wheat or that the wheat genome database is incomplete. However, in a few cases, translated protein sequences of ESTs matched up to 100 % to the known wheat proteins suggesting some amino acid substitution in these protein sequences. In one case (spot 29) (Table 4), the translated protein sequence of an EST gave a 100% match to a known wheat protein. Here, the limited number of matching peptides was insufficient to generate a significant match with the complete gene but was able to do so with the shorter EST. In fact, EST searching may be a useful approach to identify some proteins for which only limited protein sequence data can be generated.

1.4. Conclusion

In conclusion, 150 proteins separated by 2-DE were analyzed by MALDI-TOF-MS, yielding 46 unambiguous identifications. Peptide mass fingerprinting, while sensitive, faces challenges in identifying rice proteins due to incomplete genome information and insufficient data in the protein database. The study's enhanced sample preparation method for peptide mapping is deemed valuable for rice proteome analysis under these circumstances. 2-DE-based proteome analysis successfully detects and characterizes marker proteins for specific plant stress or pathological processes. Establishing wheat lemma proteome reference maps contributes to understanding spike development and aids in assigning functions to proteins. Novel techniques for high-throughput wheat proteome research are needed due to a significant percentage (30%) of blocked *N*-termini in wheat lemma proteins (Woo *et al.* 2002). These reference maps can serve as models for related crops, such as maize, barley, and sorghum, once the entire wheat and rice genome is released.

Chapter-2

Evaluation of Korean wheat quality by using proteome analysis

2.1. Diversity of novel glutenin subunits in bread Wheat (*Triticum aestivum* L.)

Abstract

Glutenin is a major determinant of baking performance and viscoelasticity, which are responsible for high-quality bread with a light porous crumb structure of a well-leavened loaf. We analysed the diversity of glutenin genes from six wheat cultivars (Korean cvs. Keumgang and Jinpum, Chinese cvs. China-108 and Yeonnon-78, and Japanese cvs. Norin-61 and Kantou-107). Glutenins contain two types of isoforms such as high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS). Glutenin fractions were extracted from wheat endosperm using Osborne solubility method. A total of 217 protein spots were separated on two-dimensional gel electrophoresis with isoelectric focusing (wide range of pH 3-10). The proteins spots were subjected to tryptic digestion and identified by matrix assisted laser desorption/ionization–time of flight mass spectrometry. HMW-GS (43 isoforms) and LMW-GS (seven isoforms) are directly responsible for producing high-quality bread and noodles. Likewise, all the seed storage proteins are digested to provide nutrients for the embryo during seed germination and seedling growth. We identified the diverse glutenin subunits in wheat cultivars and compared the gluten isoforms among different wheat cultivars according to quality. This work gives an insight on the quality improvement in wheat crop.

2.1.1. Introduction

Gluten protein composition determines the rheological characteristics (strength and extensibility) of flour dough and is the key constituent responsible for differences in end-use suitability (Butow *et al.* 2003). The gluten proteins consist of the monomeric gliadins and polymeric glutenins, where glutenins contain high and low molecular weight glutenin subunits. Wheat grain research has focused on the detailed analysis of gluten proteins to better understand those aspects of protein composition accounting for the unique properties of flour (Skylas *et al.* 2005). Variations in the types of glutenin subunits correlate with quality variations among wheat cultivars, probably by affecting the molecular weight distribution of glutenin polymers (Gupta *et al.* 1993; Gupta and Shephard 1990a, b). Although the non-gluten protein classes such as albumin and globulin occupy a smaller percentage of endosperm

protein, these proteins play critical roles in cellular metabolism, development, and responses to environment. Glutenins are made up of polypeptide chains that are cross-linked by disulfide bonds into higher level polymers. When treated with a reducing agent, glutenins dissociate into subunits of differing molecular weight: the high molecular weight subunits (HMW-GS) and the low molecular weight subunits (LMW-GS) (Payne *et al*. 1981). The glutenin consisted of two types of subunits containing LMW-GS (10-70 kDa) and HMW-GS (80-130 kDa) (Bietz and Wall 1972). When glutenins are further reduced, two types of subunits are released into high molecular weight subunit of 70-90 kDa and low molecular weight subunit of 20-45 kDa. However, actual molecular weight of glutenin calculated from derived amino acid sequences indicated 60-90 kDa, lower molecular weight rather than ever expected (Anderson and Green 1989; Anderson *et al.* 1989). Reverse-phase HPLC analysis indicates that HMW-GS to is less hydrophobic than LMW-GS. HMW-GS and LMW-GS are assumed to be cross-linked to form so-called glutenin polymers, which are among the largest molecules in nature, with molecular weights exceeding one million (Wrigley 1996). Using nullisomic-tetrasomic, nullisomic-trisomic, and ditelocentric lines of Chinese Spring, The expression of HMW-GS was controlled by gene loci at the long arms of the chromosomes 1D and 1B (Bietz and Wall 1975). The genetics of HMW-GS and their relationship to bread-making quality were conducted in details (Lawrence and Shepherd 1981; Payne *et al.* 1981). Recently, studies on the characterization of glutenins have greatly improved toward the understanding of structures and functions of HMW-GS. In particular, proteomic technology centered with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has rapidly developed rapidly and become a powerful tool for identifying storage proteins (Alberghina *et al*. 2005; Kussmann *et al*. 1997; Muccilli *et al.* 2005). Some recent investigations have focused on the tryptic peptide mapping of high molecular weight glutenin subunits (Cozzolino *et al.* 2001), but little work on measuring accurate molecular weights of the intact HMW-GS within the mixture of HMW subunits has been reported. In order to obtain accurate molecular weights of HMW-GS, we improved the resolving condition of the mixture of HMW glutenin subunits for identification by mass spectrometry, and then established a rapid MALDI-TOF-MS method to determine accurate molecular weights of single HMW glutenin subunits from mixture samples.

2.1.2. Materials and methods

2.1.2.1. Materials

Six genotypes of wheat cultivar seeds (two Korean cvs. Keumgang, Jinpum, two Chinese cvs. China-108, Yeonnon-78, and two Japanese cvs. Norin-61, Kantou-107) were used in this study for the identification of novel glutenin proteins by proteomics analysis. The seeds were collected from the field of National Institute of Crop Science, Suwon, Korea. Wheat seeds were grown in the field under low temperature (−20 to −10°C) for 4 months, then slowly increasing temperature and naturally exposed up to 28°C until harvesting. The wheat seeds were harvested for the experiment and kept in −20°C prior to the sample preparation.

2.1.2.2. Chemical reagents

All chemicals were of the highest purity and commercially available products and used without further purification. Dithioreitol (DTT), trifluroacetic acid (TFA), acetonitrile (ACN), ethylenediaminetetraacetic acid (EDTA), ammonium bicarbonate (ABC), and iodoacetamide (IAA) were purchased from Sigma (St. Louis, MI). Ultrapure water from Puris Ultrapure Water System (Mirae Co., Ltd, Korea) was used in the preparation of all solutions.

2.1.2.3. Protein extraction

We used routinely to fractionate wheat endosperm proteins, taking advantage of the solubility properties of wheat endosperm proteins in KCl, SDS, and acetone with minor modifications (Hurkman and Tanaka 2007; Osborne 1924). Fifty milligrams of flour were suspended in 200 μl of cold potassium chloride (KCl) (50 mM Tris–HCl, 100 mM KCl, 5 mM EDTA, pH 7.8). The suspension was incubated on ice for 5 min with intermittent mixing by vortexing including sonication (Sonics and Materials Inc., Newtown, USA) and centrifuged at 16,000×*g* for 15 min at 4°C (Hanil Science Industrial Co. Ltd. Korea). The pellet or KCl-insoluble fraction was suspended in 800 μl of SDS buffer (2% SDS, 10% glycerol, 50 mM DTT, 40 mM Tris–Cl, pH 6.8) and incubated for 1 h at room temperature. The resultant insoluble materials were removed by centrifugation at 16,000×*g* for 10 min at room temperature. The proteins were precipitated from the SDS buffer by adding 4 volumes of cold (kept at −20°C) acetone and incubating the mixture overnight at −20°C. After the centrifugation, the pellet was rinsed by pipetting cold acetone onto the pellet, subjected to centrifugation at 16,000×*g* for 10 min at room temperature. The supernatant was discarded by careful pipetting. The pellet (proteins including glutenin) was dried by vacuum centrifugation

(Biotron Inc., Puchon Kyunggido, Korea) and solubilized in urea buffer (9 M urea, 4% Triton X-114, 1% DTT, and 2% ampholytes) up to the final volume of 250 µl (Kamal *et al.* 2009).

2.1.2.4. Gel electrophoresis (2-DE)

Soluble proteins responsible for whole seed storage were examined by two-dimensional gel electrophoresis according to the protocol (O'Farrell 1975). Sample solutions (50 µl) were loaded on to the acidic side of the IEF gels for the first dimensional, and anodic and cathodic electrode solutions were filled in the upper and lower electrode chambers, respectively. Wheat grain proteins have been studied using 2-DE composed of the first dimension of IEF over the two pH range of pH 4–7 and 6–11 and the second dimension of SDS-PAGE (Kamal *et al.* 2009; Laemmli 1970; Woo *et al.* 2002). With the previous method, however, the separation of protein spots were not satisfactory to resolve at around the neutral (pH 4–7) pH range. Therefore, to avoid the overlapping of protein spots and to increase the resolution capacity, we adopted an IEF gel specific for pH range 3–10 in addition to the acidic and the basic pH range. SDS-PAGE in the second dimension (Nihon Eido, Tokyo, Japan) was performed with 12% separation and 5% stacking gels. Protein spots in 2-DE gels were visualized by Coomassie Brilliant Blue (CBB) R-250 staining (Woo *et al.* 2002). Each sample was run three times, and the best visualized gels were selected.

2.1.2.5. In-gel digestion and mass spectrometry analysis

According to the previous report (Kamal *et al.* 2009), selected protein spots were excised from the preparative gels, stained with Coomassie Brilliant Blue (R-250), and then washed with 100 µl distilled water. Each gel piece with protein was dehydrated by 25 mM ABC/50% ACN and washed with 10 mM DTT/0.1 M ABC. Gel pieces were dried under vacuum centrifugation, rehydrated with 55 mM IAA/0.1 M ABC for 30 min under the dark. After removing the solution, the gels pieces were vortexed with 100 mM ABC for 5 min and soaked in ACN for dehydration so that the resulting gel pieces would shrink and become an opaque-white color. The gel pieces were then dried under vacuum centrifugation. For tryptic digestion, trypsin solution (4 µl in a volume) was added in rehydrated gel particles and incubated for 45 min at 4°C and overlaid with 30 µl of 25 mM ABC (pH 8.0) to keep them immersed throughout the digestion. The gel pieces were then incubated overnight at 37ºC. After incubation, the solution was spin down and transferred to a 500-µl siliconized tube. The gel particles were resuspended in 40 µl mixture of ACN/DDW/TFA (660:330:10 µl) three times,

and again resuspended in 100% ACN, then vortexed for 30 min, respectively. The supernatant was dried under vacuum centrifugation for 2 h. In MALDI-TOF/MS (AXIMA CFR$^+$ Plus, Shimadzu, Japan) analysis, the proteins separated by 2-DE were digested in gels according to the method described (Fukuda *et al.* 2003). The samples were added in 10 µl (0.1% TFA) for the complete digestion. The digests were desalted with C_{18} Zip Tip (Millipore, Boston, MI) and subjected to the analysis by MALDI-TOF-MS.

2.1.2.6. Bioinformatics analysis

The proteins were identified by searching NCBI non-redundant database using the MASCOT program (http://www.matrixscience.com,Matrixscienc,UK). The search parameters were allowed for the modification of acetyl (K), carbamidomethyl (C), oxidation (M), propionamide (C) with peptide tolerance (±100 ppm). For MS/MS searches, the fragmentation of a selected peptide molecular ion peak was used to identify with a probability of less than 5%. Thus, MS/MS spectra with a MASCOT score higher than the significant score ($p < 0.05$) were assumed to be correct. When more than one peptide sequence was assigned to a spectrum with a significant score, the spectra were manually examined. Sequence lengths, gene names, and protein functions were identified by searching Swiss-Prot/TrEMBL database using UniProtKB (http://www.uniprot.org).

2.1.3. Results

2.1.3.1. Two-dimensional electrophoretic separation of proteins

By the conventional method, the separation of protein spots was not satisfactory at around the neutral (pH 4-7) pH range. Therefore, to avoid the overlapping of protein spots and to increase the resolution capacity, finally, we adopted an IEF gel specific for pH range 3–10 in addition to the acidic and the basic pH range. With these methods, we identified more than 250 protein spots among six cultivars by pH 3–10 range gels, which discovered about 45, 32, 38, 40, 26, and 36 protein spots, respectively (Fig. 3).These protein spot patterns were highly reproducible for at least three self-determining protein extractions. Using the 2-DE gels for pH 3–10, the qualitative variations of 36 proteins were revealed from six wheat cultivars (Fig. 1).Among them, protein spots 1, 6, 9, 16, and 17 were found in Jinpum (Fig. 1a) and protein spots 3, 4, 12, 13, 14, 15, 16, 20, 34, 38, 40, and 44 in Keumgang (Fig. 1b). In addition, protein spots 12, 13, 14, 15, 16, 19, 20, 32, 33, 37, 38, and 40 were found in Yeonnon-67

(Fig. 1c), whereas spots 17 and 18 were in China-108 (Fig. 1d). Among the Japanese wheat cultivars, protein spots 5 and 9 were in Norin-61 (Fig 1e), and protein spots 16, 19, 24, and 31 were in Kantou-107 (Fig. 1f). We identified varietal variation according to protein expression by two-dimensional electrophoresis. Out of 37 analyzed protein spots by MALDI-TOF-MS, nine expressed protein spots revealed glutenin subunits (HMW-GS and LMW-GS) in Jinpum, ten in Keumgang, four in Yeonnon-78 and China-108, and five in Norin-61 and Kantou-107, respectively (Fig. 1).

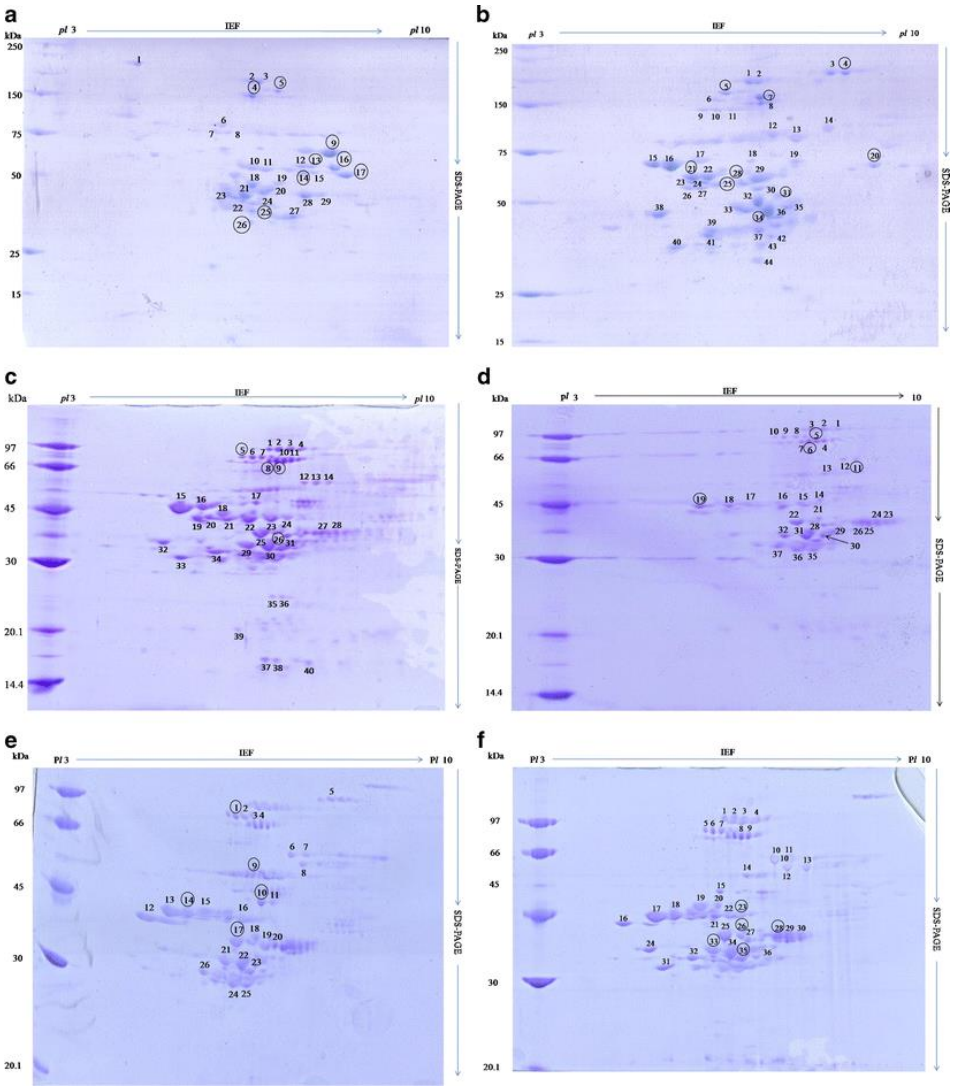

Fig. 1. Two-dimensional (2D) PAGE resolution of glutenin subunits (GSs) of six wheat cultivars (a Keumgang, b Jinpum, c China-108, d Yeonnon-78, e Norin-61, f Kantou-107). The first-dimensional gel electrophoresis (horizontal) is represented by isoelectric focusing (IEF) ranging from pH 3–10 (left pH 3; right pH 10). In the second-dimension gels (vertical) by 12% SDS-PAGE, protein spots were visualized using Coomassie Brilliant Blue R-250. The protein spots correspondence to glutenin subunits are indicated by circles. Molecular weight markers are shown on the left side with sizes.

2.1.3.2. Protein identification

The 217 protein spots were analyzed by the tryptic digestion followed by MALDI-TOF-MS. A number of proteins could not be identified by this procedure due to factors such as low resolution of mass spectrometry and the lack of genome sequence about wheat. Out of 52 glutenin proteins, using peptide fragmentation method, 13 proteins were identified in Keumgang followed by 11 in Jinpum, seven in China-108, six in Yeonnon-78, ten in Norin-61, and five in Kantou-107 (Fig. 2). Out of these, 37 selected protein spots were detected by 2-DE, and five proteins were revealed as high molecular weight glutenin subunit (HMW-GS) in Jinpum, nine in Keumgang, four in Yeonnon-78, three in China-108, and five in Norin-61 and Kantou-107 using MALDI-TOF-MS (Table 1). Some proteins identified as multiple spots differed from each other in their pls and/or Mr. Additionally, 4, 1, and 1 spots were identified as low molecular weight glutenin subunit (LMW-GS) in Jinpum, Keumgang, and China-108, respectively (Table 1). HMW-GS was identified in Keumgang (spots 31 and 34), Yeonnon-78 (spot 8) and Norin-61 (spot 1), which is directly associated with baking performance of wheat. HMW-GS was encoded by the structural gene *Glu-1* loci such as *Glu-1-2* (14–15 kDa) in Yeonnon-78 and China-108. Furthermore, HMW-GS was encoded by structural gene Glu-1 loci as *Glu-1B* (79.4 kDa) and *Glu-1B* (77.2 kDa) and *Glu-1R* (77.3 kDa) in Yeonnon-78 and Norin-61, respectively. LMW-GS proteins were encoded by LMW-GS gene (26–40 kDa) in Jinpum, Keumgang and China-108 (Table 1). Interestingly, we identified the different types of glutenin subunits in wheat such as y-type (19.6 kDa from Aegilops ventricosa, 1.5 kDa from Leymus racemosus, and 17.2 kDa from Triticum aestivum) and s-type (27.7 kDa from Triticum aestivum and 34.7 kDa from Aegilops tauschii) in our experiment (Table 1 and Fig. 3).

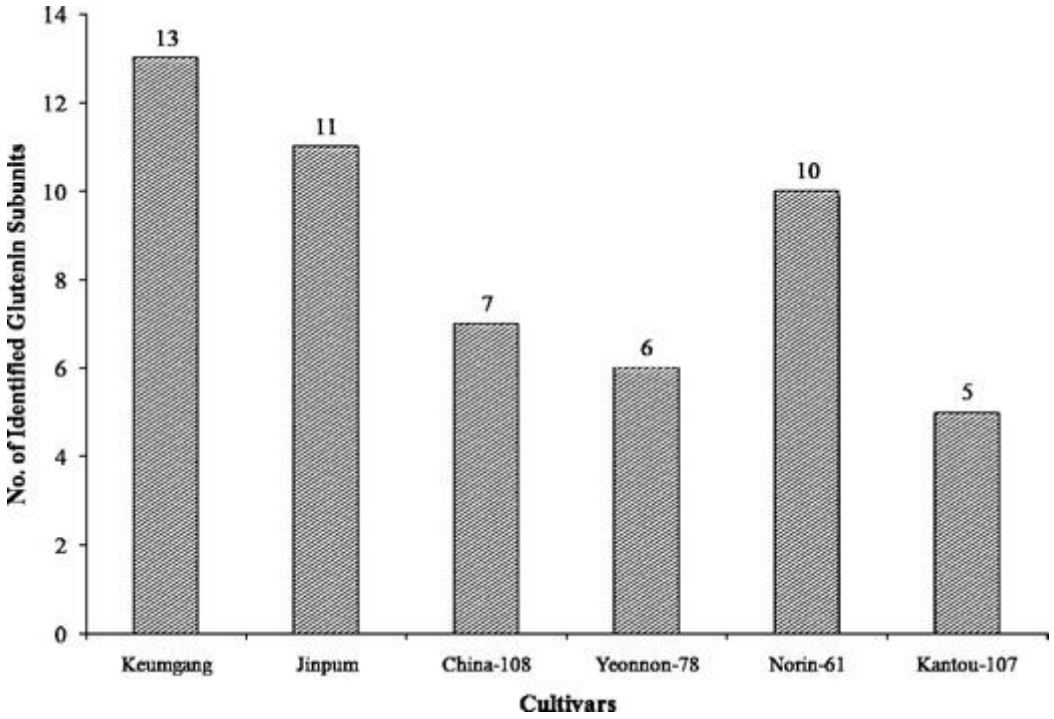

Fig. 2. Distribution of total identified glutenin subunits (GSs) protein among wheat cultivars

Table 1. List of identified glutenin subunits (GSs) protein from six wheat cultivars including name of gene by using MALDI-TOF-MS coupled to bioinformatics

SN	Identified Protein	Mr	p^I Value	Species	Gene identifier	Score	SC (%)	GN	CN
4	Y-type HMW- glutenin subunit	19,683	8.64	*Aegilops ventricosa*	gi|7188718	55	18	–	Jinpum
	HMW- glutenin subunit	14,991	9.17	*Triticum aestivum*	gi|32328619	52	37	HMW-GS	
5	HMW-glutenin subunit	19,908	8.85	*Triticum aestivum*	gi|24474926	73	23	HMW-GS	
	Y-type HMW glutenin subunit	19,683	8.64	*Aegilops ventricosa*	gi|7188718	71	24	–	
9	LMW- glutenin subunit group 3 type II	26,718	8.21	*Triticum aestivum*	gi|17425184	32	20	LMW-GS	
13	Glutenin, high molecular weight subunit PC237	4,058	8.2	*Triticum aestivum*	gi|121451	18	33	–	
14	Low-molecular-weight glutenin subunit	30,679	8.69	*T. turgidum*subsp. *polonicum*	gi|124109356	17	6	LMW-GS	
16	LMW-glutenin subunit -S13 precursor	34,733	9.08	*Aegilops tauschii*	Q6J6U8_AEGTA	20	14	–	
17	LMW-Glutenin subunit	40,994	9.04	*Triticum aestivum*	GLTA_WHEAT	16	3	–	
25	LMW- glutenin	32,501	8.82	*T.turgidum*subsp. *dicoccoides*	gi|53854906	39	25	–	
26	HMW-glutenin PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	13	61	–	
4	High-molecular-weight glutenin subunit	15,006	8.95	*T. aestivum*subsp. *spelta*.	Q7XZA8_WHEAT	25	58	GLU-1–2	Keumgang
5	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	gi|7188718	38	17	–	
	HMW glutenin subunit 1By16	79,420	8.75	*Triticum aestivum*	gi|146261042	34	7	–	
	HMW glutenin subunit Dty10	27,040	8.2	*Aegilops tauschii*	gi|46981764	33	12	–	
7	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	Q9M5N3_AEGVE	33	11	–	
20	Y-type HMW- glutenin subunit	1,572	8.53	*Leymus racemosus*	gi|71159594	14	93	–	
21	LMW-glutenin subunit group 4 type II	38,417	8.89	*Triticum aestivum*	gi|17425188	33	15	LMW-GS	
	S-type low molecular weight glutenin L4-55	27,777	8.51	*Triticum aestivum*	Q6J160_WHEAT	30	15	–	
25	Y-type HMW-glutenin subunit	1,572	8.53	*Leymus racemosus*	Q1G7F6_9POAL	14	93	–	
28	HMW-Glutenin subunit PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	13	41	–	
31	High molecular weight glutenin	1,007	8.53	*Triticum aestivum*	Q308Z8_WHEAT	12	87	GLU-DY	
34	Glutenin high molecular weight subunit	19,908	8.85	*Triticum aestivum*	Q8H0L3_WHEAT	20	34	HMW-GS-DY	
36	Glutenin, high molecular weight subunit PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	10	41	–	
5	Glutenin, high molecular weight subunit PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	11	12	–	Yeonnon-78

8	High-molecular-weight glutenin subunit	14,973	9.17	*Triticum aestivum*subsp. *spelta*	gi|32328625	29	30	GLU-A1-2	
	High-molecular-weight glutenin subunit	14,991	9.17	*Triticum aestivum*	Q7XZB8_WHEAT	44	51	GLU-A1-2	
	HMW glutenin subunit Dty10	27,040	8.2	*Aegilops tausch*	Q6PMI8_AEGTA	33	28	–	
9	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	Q9M5N3_AEGVE	30	19	–	
26	High-molecular-weight glutenin subunit	14,991	8.95	*Triticum aestivum*subsp. *spelta*	Q7X6P8_WHEAT	15	19	GLU-A1-2	
	High-molecular-weight glutenin subunit	14,991	9.17	*Triticum aestivum*	Q7XZB8_WHEAT	44	51	GLU-A1-2	
	HMW glutenin subunit Dty10	27,040	8.2	*Aegilops tausch*	Q6PMI8_AEGTA	33	28	–	
9	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	Q9M5N3_AEGVE	30	19	–	
26	High-molecular-weight glutenin subunit	14,991	8.95	*Triticum aestivum*subsp. *spelta*	Q7X6P8_WHEAT	15	19	GLU-A1-2	
5	High-molecular-weight glutenin subunit	15,046	8.7	*Triticum aestivum*subsp. *spelta*	gi|32328663	16	12	GLU-A1-2	China-108
6	Glutenin, high molecular weight subunit PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	11	61	–	
	High molecular weight gluteni y-type	17,281	8.91	*Triticum aestivum*	gi|220900283	20	9	–	
11	Glutenin, high molecular weight subunit PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	21	69	–	
	High-molecular-weight glutenin	17,860	5.35	*Triticum aestivum*	JC4966	19	24	–	
19	S-type low molecular weight glutenin L4-55	27,777	8.51	*Triticum aestivum*	Q6J160_WHEAT	38	7	–	
	LMW-glutenin subunit group 4 type II	33,456	8.71	*Triticum aestivum*	Q8W3W3_WHEAT	36	6	LMW-GS	
1	HMW glutenin subunit 1By8	77,297	8.76	*Triticum turgidum*subsp. *durum*	Q84TG6_TRITU	36	11	GLU-1B	Norin-61
	Glutenin high molecular weight subunit	19,908	8.85	*Triticum aestivum*	gi|24474926	56	33	HMW-GS-DY	
	Glutenin, high molecular weight subunit 12	70,824	7.64	*Triticum aestivum*	GLT3_WHEAT	25	9	–	
	HMW glutenin subunit Dty10	27,040	8.2	*Aegilops tauschii*	Q6PMI8_AEGTA	41	17	–	
	High molecular weight glutenin subunit y	77,325	8.94	*Triticum aestivum*	gi|14329763	44	13	GLU-1R	
	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	Q9M5N3_AEGVE	48	24	–	
9	Glutenin, high molecular weight subunit PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	20	41	–	
10	Glutenin, high molecular weight subunit PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	11	12	–	
14	High-molecular-weight glutenin	17,860	5.35	*Triticum aestivum*	JC4966	18	37	–	
17	Glutenin, high molecular weight subunit PC237	4,058	8.2	*Triticum aestivum*	GLT2_WHEAT	10	41	–	
23	Glutenin, high molecular weight subunit PC256	10,889	8.18	*Triticum aestivum*	GLT1_WHEAT	19	29	–	Kantou-107

26	High-molecular-weight glutenin	17,860	5.35	*Triticum aestivum*	JC4966	17	25	–	
28	High-molecular-weight glutenin subunit	3,053	9.98	*Aegilops kotschyi*	gi|225380772	14	82	HMW-GS	
33	Glutenin, high molecular weight subunit PC256	10,889	8.18	*Triticum aestivum*	GLT1_WHEAT	8	23	–	
35	High-molecular-weight glutenin	17,860	5.35	*Triticum aestivum*	JC4966	22	34	–	

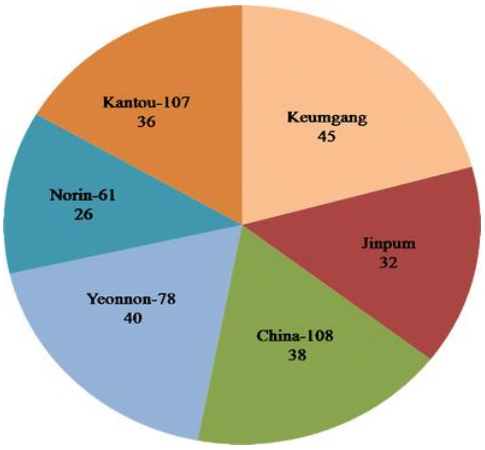

Fig. 3. Distribution of total detected protein spots by two-dimensional electrophoresis from six wheat cultivars

2.1.4. Discussion

Throughout the world, many Asian countries have recently launched programs to quality development with balanced nutrients and good bread and noodles made of wheat. Our research has focused on identifying new proteins to affect the wheat quality. Using 2-DE and MALDI-TOF/MS technique, the proteomic profiles of seeds were analyzed from the relatively high-quality cultivars among Jinpum, Keumgang, Yeonnon-78, China-108, Norin-61, and Kantou-107. Two thousand seventeen protein spots were chosen based on protein spot intensity among six wheat cultivars. By the database search, the selected proteins were separated into two functional groups. The first contains HMW-GS proteins to directly affect the baking performance and viscoelasticity. The second group contains LMW-GS proteins to affect the bread-making quality, which are associated with HMW-GS as major storage proteins.

Most of wheat seed proteins are accumulated as protein bodies in the endosperm and embryo or in the aluerone layer. Wheat seed storage proteins, composing of HMW-GS, LMW-GS, and gliadin, are the main contributors to wheat flour quality to affect food preference (Tanaka et al. 2005). The HMW-GSs are encoded by genes at the homologous loci, *Glu-A1*, *Glu-B1*, and *Glu-D1*, which are located in the long arm of the homologous group 1 chromosome, which are 1AL, 1BL, and 1DL, respectively (Payne *et al*. 1982). HMW-GS plays critical roles in bread-making quality (Payne *et al.* 1984), and allelic variations existed at each of the *Glu-1* loci with tightly linked genes encoding x-type and y-type HMW-GS (Payne 1987).

We identified Glu-1-2 alleles in Yeonnon-78 and China-108, which originated from Chinese wheat cultivars. Five *Glu-A1-2* alleles were identified in 19 informative sites, which suggested a polyphyletic origin of the A- and B-genomes of hexaploid wheat from Asian wheat (Blatter et al. 2004). The cultivars such as Keumgang, Yeonnon-78, and Norin-61 have *Glu-Dy* alleles similar to the previous report (Giles and Brown 2006), which has undergone relatively rapid change since polyploidization. Duplications and deletions of these motifs are responsible for the allelic variation at the *Glu-1R* locus. Orthologous genes (from different genomes) were more close than paralogous genes (x- and y-type), supporting the hypothesis of gene duplication before Triticeae speciation. Differences in the number and position of cysteine residues identified alleles in which the wheat cultivars are associated with good dough quality (De Bustos and Jouve 2003).Two tightly linked genes encode a lower molecular mass y-type subunit and a higher molecular mass x-type subunit

at each locus, respectively. These two genes share a similar primary structure: a signal peptide (cleaved off during maturing), conservative N- and C-terminal domains, which contain most of the cysteine residues present in the HMW glutenin subunits, and a central repetitive domain constituted of tripeptides (only in x-type), hexapeptides, and nonapeptides, which are similar to our experiment (Shewry *et al.* 1992).

Low molecular weight (LMW) GSs ranging from about 30 to 60 kDa are associated with bread-making quality (Payne 1987). LMW-GSs are encoded by the *Glu-3* loci on the short arms of homologous group 1 chromosomes. The *Glu-3* loci consist (Gupta and Shephard 1990a, b) of a multigene family, estimated to include 30-40 genes (Cassidy *et al.* 1998). However, little is known about the roles of LMW-GSs in bread-making quality. Almost all LMW-s are, however, not associated with any cloned gene. The characterization of gene(s) encoding the LMW glutenin components associated with good bread-making quality of "Keumgang" would be helpful for elucidating how the components improve dough properties. Thus, we demonstrated that the detailed characterization of glutenin components encoded by alleles from six cultivars is possible to separate on 2D-gel. The technique is a good tool for the characterization of HMW and LMW glutenin complexes in genetic analyses such as segregation of products encoded by *Glu-1* alleles. Finally, the selection of seeds carrying both of HMW-GS and LMW-GS by wheat breeders should lead to the development of new varieties of wheat with improved bread-making qualities.

In conclusion, rapid and accurate differentiation of glutenin subunits is important in wheat quality improvement. To date, 2D-PAGE has been the most popular method to detect glutenin proteins compositions of wheat cultivars. In addition, MALDI-TOF/MS technology has been established for characterization of wheat storage proteins accompanying the development of wheat proteomics (An *et al.* 2006; Pei *et al.* 2007; Wrigley 1996). The *Glu-B1* proteins are highly variable, and the variants are often related to different quality attributes and represent a group of biochemical factors that are not yet fully utilized for wheat quality improvement. Since these variants often have similar molecular weights, it is usually difficult to differentiate them by traditional SDS-PAGE methods. The current study indicated that MALDI-TOF-MS is a powerful technique for rapid identification of HMW-GS allele diversity at the *Glu-B1* locus (Li *et al*. 2009). Therefore, the accurate molecular masses of the particular glutenin subunit could be obtained rapidly as performed throughout this study. Combining the traditional gel with MALDI-TOF/MS may

provide an alternative tool to accelerate quality-improvement procedures of wheat cultivars. In addition, this basic proteomic tool can be particularly useful for accurately identifying desirable glutenin subunits in the early generations of hybridization when some candidate subunits are expected to transfer from one wheat cultivar to others.

2.2. Influence of allelic variations in glutenin on the quality of bread and white salted noodles made from Korean wheat cultivars

Abstract

Dough rheological properties and end-use quality were evaluated to determine the effects of *Glu-1* and *Glu-3* alleles on those characteristics in Korean wheat cultivars. SDS-sedimentation volume based on protein weight was positively correlated with mixograph parameters and maximum height of dough and also positively correlated with bread volume, crumb firmness and springiness of cooked noodles. Protein content was negatively correlated with optimum water absorption of noodle dough, lightness of noodle dough sheet and hardness and cohesiveness of cooked noodles. Within *Glu-1* loci, 1 or 2* subunit and 5 + 10 subunits showed longer mixing time, higher maximum dough height and larger bread volume than other alleles. Cultivars with 13 + 16 subunits at *Glu-B1* locus showed higher protein content and optimum water absorption of mixograph than cultivars with 7 + 8 subunits. At *Glu-3* loci, *Glu-A3d* showed longer mixing time than *Glu-A3e*, and *Glu-B3d* and *Glu-B3h* had stronger mixing properties than *Glu-B3i*. Glu-B3h had higher bread volume and hardness of cooked noodles than *Glu-B3d*. *Glu-D3a* had lower protein content than *Glu-D3c*, and *Glu-D3b* showed stronger mixing properties than *Glu-D3a*. *Glu-D3c* showed lower hardness of cooked noodles than others.

2.2.1. Introduction

Glutenins, the major seed storage proteins in wheat, consist of high-molecular-weight glutenin subunits (HMW-GS, 80-130 kDa) and low-molecular-weight glutenin subunits (LMW-GS, 30-50 kDa) which are separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Gianibelli *et al.* 2001). Although HMW-GS comprise only about 10% of wheat gluten, they are the key factors in bread-baking process as the major determinants of dough elasticity. LMW-GS represent approximately 40% of wheat gluten and play a major role in determining dough resistance and extensibility, and to a lesser degree strength, both directly and indirectly via their interaction with HMW-GS.

HMW-GS are encoded by *Glu-A1*, *Glu-B1* and *Glu-D1* on the long arm of chromosome 1 (Payne and Lawrence 1983). Payne group proposed a nomenclature system of HMW-GS and assigned scores to each identified *Glu-1* allele, which made it possible to predict the approximate bread

making quality of wheat cultivars (Payne 1987). Shewry *et al.* (1992) proposed that wheat varieties with good bread baking quality might require allelic subunits 1 or 2* on *Glu-A1* locus, 17 + 18 or 7 + 8 on *Glu-B1* locus, and 5 + 10 on *Glu-D1* locus.

LMW-GS are encoded by *Glu-A3*, *Glu-B3* and *Glu-D3* located on the short arm of chromosome 1 (Jackson *et al.* 1983). LMW-GS have not been studied so intensively as HMW-GS due to their complex banding patterns observed and the mobility which overlaps with gliadins (Gupta and Shepherd 1990; Jackson *et al.* 1996). For these reasons, the study on the function of LMW-GS and the effect of their allelic variations on the quality of wheat has received far less attention than that of HMW-GS. Several studies have been carried out to evaluate the effects of *Glu-3* allelic variations on dough properties (Branlard *et al.* 2001; Eagles *et al.* 2001; Gupta *et al.* 1994; Killermann and Zimmermann 2000; Maucher *et al.* 2009; Metakovsky *et al.* 1990). The interactive effects of *Glu-1* and *Glu-3* loci on flour and end-use quality have been also estimated since both HMW-GS and LMW-GS are useful genetic markers for quality improvement of wheat (Flæte and Uhlen 2003; He *et al.* 2005; Kolster et al. 1991; Liu *et al.* 2005; Luo *et al.* 2001; Nagamine *et al.* 2000; Nieto-Taladriz *et al.* 1994; Tabiki *et al.* 2006; Zhang *et al*. 2007, 2009).

Korean wheat breeding program has focused on improving grain yield and early maturation and about 30 cultivars have been developed since 1970s. Recently, importance of end-use quality of wheat comes across to the surface of the program and developing gluten quality associated with bread-baking and noodle-making process has received more attention by wheat breeders than ever. Most Korean wheat cultivars and advanced lines have 2.2 + 12 subunits in *Glu-D1* locus and they showed inferior noodle color to commercial noodle wheat flours and lower loaf volume and harder crumb firmness than commercial bread flours in spite of the similar protein content (Park *et al.* 2005, 2006). However, there was little information about the association of allelic variations in glutenin including HMW-GS and LMW-GS with end-use properties of Korean wheat cultivars. Therefore, this study was conducted to elucidate the relationship between dough rheological properties and end-use characteristics of Korean wheat cultivars through the comparison with commercial flours and imported wheat flours, to determine the effects of *Glu-1* and *Glu-3* alleles on those characteristics of Korean wheat cultivars, and to provide useful information for improving wheat quality in Korean wheat breeding programs.

2.2.2. Materials and methods

2.2.2.1. Plant materials

Twenty six Korean wheat cultivars, which are representative wheat cultivars breed since 1970's, were harvested in Iksan (Winter Cereal & Forage Crop Research Division, National Institute of Crop Science, RDA) in 2008. The seeds were sawn on October 25th and mean temperature (8.8°C) and precipitation (472 mm) were similar to the mean values for 10 years. Korean wheat cultivars were milled using Bühler experimental mill, and flour of about 60% extraction was prepared by blending millstreams. Four imported wheat flours including soft white (SW), Australian standard white (ASW), hard red winter wheat (HRW) and dark northern spring (DNS), and three commercial wheat flour suitable for bread baking (Com1), making wet noodles (Com2) and dry noodles (Com3) were obtained from Samyang Milmax (Asan, Korea) were used for this study.

2.2.2.2. Glutenin subunit compositions

Glutenin subunit compositions were evaluated according to the protocol of Singh et al. (1991) with some modifications reported by Peña et al. (2004). HMW-GS were classified using the nomenclature of Payne and Lawrence (1983). *Glu-A3* and *Glu-D3* alleles were evaluated according to the nomenclature of Gupta and Shepherd (1990). Allelic variations of *Glu-B3* were evaluated by combining their corresponding allelic variations of gliadin according to the nomenclature of Jackson *et al.* (1996).

2.2.2.3. Analytical methods

Particle size distribution of flour was determined using LS13320 laser diffraction particle size analyzer (Beckman Coulter, Inc, CA, USA). Wheat flour (5 g) weighed was transferred into laser diffraction particle size analyzer's dispersion tube and its size was measured. The determination of amylose content was performed according to the procedure described by Gibson *et al.* (1997) using an enzymatic assay kit (Megazyme Pty., North Rocks, Australia). Moisture and protein content of wheat flour were determined according to AACC methods, 44-15A and 46-30 (AACC 2000). The SDS-sedimentation test was performed on a constant protein (300 mg) basis according to the procedure of Baik *et al.* (1994). Optimum water absorption, mixing time and mixing tolerance of wheat flour were determined using a 10 g mixograph (National Mfg., USA) according to the approved method, 54-40A (AACC 2000). Rheofermentometer (Chopin, France) dough

development and gas retention of flour were determined according to the procedure of Czuchajowska and Pomeranz (1993).

2.2.2.4. Bread baking

Bread was baked according to the straight-dough methods described by Finney (1984). The ingredients of baking formula consisted of 100 g (14% moisture basis) of flour, 6 g of sugar, 3 g of shortening, 1.5 g of salt, 5.0 g of fresh yeast, 50 mg of ascorbic acid, and 0.25 g of barley malt (about 50 DU/g, 20°C). The optimum water absorption and mixing time were determined by the feel and appearance of the dough during the mixing. The dough was fermented in a cabinet at 30°C and 85% relative humidity for 70 min with two punches and a proof period of 60 min, and then baked at 210°C for 18 min. Bread loaf volume was measured by rapeseed displacement in a graduated chamber. After cooling for 2 h at room temperature, a slice 2.0 cm thick was cut from the center portion of the bread. Loaf volume was measured immediately by rapeseed displacement and weighted after the bread was taken out of the oven. Firmness of the bread crumb was evaluated with a compression test using TA-XT2 Texture Analyser (Stable Micro Systems, England). The slice was placed on a flat metal plate and compressed to 25% of its thickness at the speed of 1.0 mm/s using a plastic plunger with a flat surface of 2.0 cm diameter.

2.2.2.5. Preparation of white salted noodles

White salted noodles were prepared with optimum water absorption percent of noodle dough. The optimum water absorption percent for making white salted noodles was determined based on appearance and sheeting and handling properties of the dough during the noodle making process by experienced personnel, through trial and error. Com2 and 3, which required 34% absorption to make uniform, smooth and nonsticky dough, were used as a reference to be compared with other flours during the determination of optimum water absorption percent.

Flour (100 g, 14% mb) was mixed with the predetermined amount of 2.0% sodium chloride solution in a pin mixer (National Mfg., USA) for 4 min, with a head speed of 86 rpm. The concentration of sodium chloride solution for making noodles with different absorption was adjusted to have 2.0% sodium chloride in the noodle dough. Dough was passed through the rollers of a noodle machine (Ohtake Noodle Machine Mfg., Japan) at 8 rpm and a 3 mm gap; dough was folded and put through the sheeting rollers. The folding and sheeting were repeated twice. The dough sheet was rested for 1 h and then put through the sheeting rollers three times at progressively

decreasing gaps of 2.40, 1.85 and 1.30 mm. A piece of noodle sheet was placed in plastic bags for determination of color. Lightness of the dough sheet was measured by Minolta CM-2002 (Minolta Camera Co., Japan) with an 11 mm measurement aperture. The rest of the dough sheet was cut through no. 12 cutting rolls into strips about 30 cm in length. The rest of the dough sheet was cut through no. 12 cutting rollers into noodle strands of about 30 cm in length, with a 0.3 × 0.2 cm cross section.

2.2.2.6. Textural properties of cooked noodles

Raw noodles (20 g) were cooked at the determined cooking time in 500 ml of boiling distilled water for 18 min and then rinsed with cold water. Two replicates of cooked noodles were evaluated by texture profile analysis (TPA) using TA-XT2 Texture Analyser (Stable Micro Systems, England) within 5 min after cooking. One set of five strands of cooked noodles was placed parallel on a flat metal plate and compressed crosswise twice to 70% of their original height using a 3.175 mm metal blade at the speed of 1.0 mm/s. From force-time curves of the TPA, hardness, springiness and cohesiveness were determined according to the description of Park and Baik (2002).

2.2.2.7. Statistical analysis

Statistical analysis of data was performed by SAS software (SAS Institute, NC, USA) using Fisher's least significant difference procedure (LSD), analysis of variance (ANOVA) and Pearson correlation coefficient. Differences were considered significant at $p < 0.05$, unless otherwise specified. All data were determined at least in duplicate and all were averaged.

2.2.3 Results

2.2.3.1. Physicochemical and rheological properties

Average particle size of flour of 26 Korean wheat cultivars ranged from 57.8 to 96.5 μm (Table 1). Soft white wheat (SW) showed the finest particle size of flour in imported wheat flours. Commercial flour for bread baking (Com1) had slightly coarser particle size than other commercial flours. Chungkye, Dahong, Jinpoom, Milsung, Namhae, Ol, Olgeuru, Saeol and Uri showed similar average particle size of flour to SW and commercial flours. Average particle size of flour of other Korean wheat flours was similar to that of Australian standard white (ASW), hard red winter (HRW) and dark northern spring (DNS). Amylose content of Korean wheat flours ranged

from 24.7 to 26.8%, which no difference in amylose content in Korean wheats. No difference in amylase content between imported wheat flours and commercial wheat flours, except for ASW wheat flour (22.4%). Protein content and SDS-sedimentation volume of 26 Korean wheat cultivars ranged from 8.4 to 14.0% and from 18.5 to 56.0 ml, respectively. Protein content of DNS and Com1 was higher than that of other imported and commercial wheat flours. Compared to commercial wheat flours, Joeun and Jopoom showed similar protein content and SDS-sedimentation volume of Com1 and Baekjoong, Chungkye, Olgeuru, Saeol were similar to that of Com2 and 3.

Table 1. Physicochemical properties and rheological properties of Korean wheat cultivars, imported wheat and commercial flours

Flour	Particle size (μm)	Amylose (%)	Protein (%)	SDSS[a] (ml)	Mixograph[b]			Rheofermentometer	
					Abs (%)	Time (min)	Tol (mm)	Max. dough height (mm)	Retention coefficient of dough (%)
Korean wheat									
Alchan	80.6	26.8	9.3	55	57.5	5.3	20	39.1	92.8
Alchan	80.6	26.8	9.3	55	57.5	5.3	20	39.1	92.8
Anbaek	90.7	24.7	12	45.5	59	2.4	11	32.3	92.9
Baekjoong	91.2	25	10.2	45.5	59.5	3.6	14	35.6	93.2
Chungkye	63.6	25.5	10.7	39	57.5	3	10.5	25.4	95.1
Dahong	58	26.6	9.9	18.5	55.5	2.1	9.5	25.2	92.9
Eunpa	90.1	25.5	12.3	41.5	62.5	3.5	12.5	30.6	93.6
Geuru	82.5	24.8	13.9	20.5	60	2.3	6.5	19.8	93.6
Gobun	86.1	24.7	11.5	53.5	58	3.8	24	31.3	95.3
Hanbaek	95.5	25.8	12	44.5	57.5	3.7	16	32.9	93.2
Jeokjoong	95.5	24.7	10.5	48.5	60	3.4	11.5	36.9	94.2
Jinpoom	60.6	26.1	9.9	46.5	57.5	3.9	21.5	31	92.8
Joeun	96.5	25.5	14	44.5	62.5	3	19	26.7	93.9
Jokyung	86.7	24.9	10.8	54.5	59.5	4	22.5	51.5	95.6
Jonong	75.6	24.8	13.1	30.5	60.5	3.5	12	36.6	92.6
Jopoom	79	24.5	14	47.5	63.5	3.5	14.5	30.2	93.5
Keumkang	86.2	24.9	11.9	56	60.5	4.1	22	45.9	96.9
Milsung	63.2	25.7	9.8	18.5	53	2.4	11	30.7	93.5
Namhae	58.5	26.2	10.9	26	59.5	3.4	13	34.7	92.6
Ol	62.6	24.8	10.1	26.5	58.5	2.4	10.5	27.2	93.8
Olgeuru	67	25.1	9.9	37.5	57.5	3.4	16	26.4	94.7
Saeol	61	24.8	10.3	45.5	57.5	2.8	14	27.3	92.1
Seodun	89.5	26.5	9.3	55.5	56.5	2.9	19	24.3	94
Sukang	95.2	24.9	13.2	52.5	59	3.3	9.5	35.4	94.3

Tapdong	81.4	24.7	11.8	45.5	60.5	5.6	24	48.9	95.7
Uri	57.8	26.3	8.4	25.5	56.5	4.1	17	36.2	94.2
Younbaek	91.3	25.4	11.2	38.5	57.5	3.4	19.5	36.6	94
Imported wheat flour[c]									
SW	60.2	26.5	9.4	32.5	53	1.7	17.5	32.9	93.3
ASW	83.4	22.4	11.4	44	59.5	3.9	16.5	32.9	93.4
HRW	91.9	24.1	12.4	41	62	4.4	19.5	47.2	95.3
DNS	94.2	24.1	15.1	51	66	5	19	52.3	97.9
Commercial flour[d]									
Com1	66.6	23.4	13.8	42.5	65	4.5	28.5	50.3	97.7
Com2	57.3	23.5	10.6	38.5	60	3.2	24	29.3	96.5
Com3	56.2	23.7	10.4	40.5	60.5	3.6	19	39.1	96.7
LSD[e]	0.9	2.2	0.2	0.8	0.7	0.1	0.4	3.1	1.2

[a]SDSS SDS sedimentation volume based on constant protein content (300 mg)

[b]Abs water absorption, Time mixing time, Tol mixing tolerance at 7 min

[c]SW soft white, ASW Australian standard white, HRW hard red winter, DNS dark northern spring

[d]Com1 commercial flours for bread baking, Com2 and 3 commercial flour for wet and dry noodles, respectively

[e]Least significant difference (P = 0.05). Differences between two means exceeding this value are significant

Mixograph parameters are generally used to compare wheat flours for their difference in protein quality. Optimum water absorption of mixograph increased from 53.0 to 63.5% for Korean wheat cultivars as protein content increased ($r = 0.728$, $p < 0.001$). Optimum water absorption of DNS and Com1 was higher than those of other imported, commercial flours and Korean wheat cultivars. Compared to commercial and imported wheat flours, Geuru, Jeokjoong, Jonong, Keumkang and Tapdong showed similar optimum water absorption of Com2 and 3 and Baekjoong, Jokyung and Namhae were similar to that of ASW. Mixing time of Korean wheat flours was longer (2.1–4.1 min) than SW (1.7 min) but shorter than Com1, HRW and DNS (4.5, 4.4 and 5.0 min, respectively), except in Alchan and Tapdong (5.3 and 5.6 min, respectively). Mixing tolerance of Com1 and 2 (>24.0 mm) was higher than that of Com3 and imported wheat flours (16.5–19.5 mm). Mixing tolerance of Korean wheat flours (6.5–19.5 mm) was lower than imported wheat flours, except in Alchan, Gobun, Jinpoom, Jokyung, Keumkang, Tapdong (>20.0 mm). Mixing time and tolerance of Korean wheat flours were positively correlated with SDS-sedimentation volume ($r = 0.512$, $P < 0.01$ and $r = 0.600$, $P < 0.001$, respectively, Fig. 1a and b).

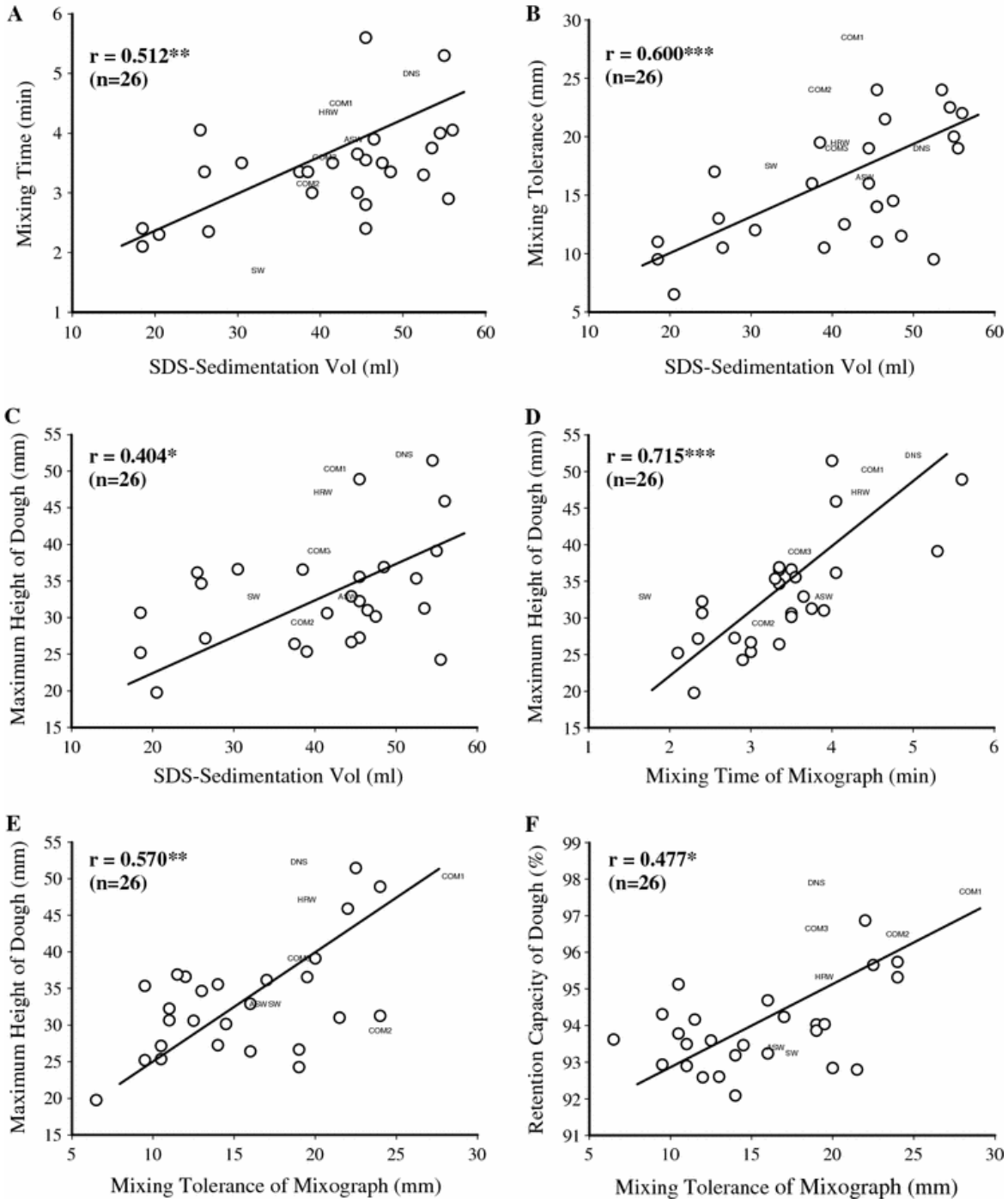

Fig. 1. The relationships between SDS-sedimentation volume and dough rheological properties, including mixing time (a), mixing tolerance (b) and maximum height during dough development (c), between mixing time of Mixograph and maximum height during dough development (d), between mixing tolerance of Mixograph, maximum height during dough development (e) and gas retention capacity of dough (f) from 26 Korean wheat flours

Before baking, dough development, gas formation and gas retention were predicted by using Rheofermentometer. The maximum dough height of Jokyung, Keumkang and Tapdong (45.9-51.5 mm) was similar to HRW, DNS and Com1 (47.2-52.3 mm), but other Korean wheat flours (19.8–39.1 mm) showed lower maximum dough height than those commercial and imported wheat flours. The gas retention coefficient of dough, which represents the amount of gas retained by dough at the end of fermentation, in Jokyung, Keumkang and Tapdong (95.6-96.9%) was higher than other Korean wheat flours (<95.3%). The gas retention coefficient of Jokyung, Keumkang and Tapdong was similar to that of HRW and Com2 and 3, in spite of lower than DNS and Com1 (97.9 and 97.7%, respectively). Anbaek, Baekjoong, Eunpa, Hanbaek, Jinpoom, Jopoom, Milsung, Namhae and Sukang showed similar dough rheological properties to ASW and SW. Maximum dough height of Korean wheat cultivars was positively correlated with SDS-sedimentation volume ($r = 0.404$, $p < 0.05$ Fig. 1c) and mixing time and tolerance of mixograph ($r = 0.715$, $p < 0.001$ and $r = 0.570$, $p < 0.01$, respectively, Fig. 1d and e). Gas retention capacity of dough was positively correlated with mixing tolerance of mixograph ($r = 0.477$, $p < 0.05$, Fig. 1f), although there was no significant relationship between protein content and dough properties measured by rheofermentometer in Korean wheats.

2.2.3.2. Bread and noodle qualities

Korean wheat flours showed lower loaf volume (<885 ml) and higher firmness (>1.0 N) than wheat flours suitable for baking bread, DNS and Com1, which had >997 ml in loaf volume and <0.9 N in crumb firmness (Table 2). Tapdong showed higher loaf volume than other Korean wheat flours (885 ml and 1.1 N, respectively), which was comparable to loaf volume and crumb firmness of HRW (917 ml and 1.2 N, respectively). Crumb firmness decreased as loaf volume increased in Korean wheat cultivars, imported and commercial flours. In Korean cultivars, loaf volume and crumb firmness were significantly correlated with mixing time of mixograph and maximum height during dough development (Fig. 2a and b). Those bread quality parameters were also significantly correlated with optimum water absorption of mixograph ($r = 0.442$, $p < 0.05$ and $r = -0.614$, $p < 0.001$, respectively). Crumb firmness was also negatively correlated with SDS-sedimentation volume ($r = -0.546$, $P < 0.01$), but there was no significant relationship between protein content and bread parameters in Korean wheat cultivars.

Table 2. Characteristics of bread and white salted noodles prepared from Korean wheat cultivars, imported wheat and commercial flours

Cultivar	Bread		White salted noodle				
	Loaf volume (ml)	Firmness (N)	Dough sheet		Cooked noodles		
			Absorption (%)	Lightness	Hardness (N)	Springiness (Ratio)	Cohesiveness (Ratio)
Korean wheat							
Alchan	860	1.5	35	84.43	4.97	0.92	0.63
Anbaek	820	1.6	33	80.16	4.97	0.92	0.63
Baekjoong	798	1.5	36	82.96	4.23	0.93	0.63
Chungkye	753	1.3	35	83.53	4.26	0.93	0.62
Dahong	818	1.7	35	84.47	4.8	0.91	0.63
Eunpa	808	1.7	33	80.45	5.87	0.92	0.64
Geuru	775	2.1	35	79.67	5.63	0.91	0.63
Gobun	758	2.2	34	80.43	5.84	0.92	0.63
Hanbaek	820	1.6	34	82.61	4.24	0.94	0.64
Jeokjoong	768	1.7	35	83.47	4.78	0.93	0.64
Jinpoom	763	1.5	35	83.47	4.15	0.93	0.62
Joeun	793	1.6	33	79.43	6.21	0.92	0.63
Jokyung	875	1.2	34	83.11	5.46	0.92	0.63
Jonong	865	1.4	33	79.63	6.3	0.93	0.63
Jopoom	800	1.2	31	80.42	6.33	0.93	0.64
Keumkang	848	1	33	83.09	5.12	0.93	0.64
Milsung	688	2.1	35	84.9	3.81	0.91	0.62
Namhae	838	1.5	35	83.6	4.69	0.93	0.63
Ol	825	1.7	35	83.8	4.91	0.92	0.63
Olgeuru	813	1.7	35	83.33	4.19	0.92	0.63
Saeol	763	1.4	35	83.75	4.66	0.93	0.63
Seodun	678	1.9	35	84.19	4.21	0.92	0.62
Sukang	793	1.3	34	81.69	4.3	0.95	0.63
Tapdong	885	1.1	33	81.45	6.02	0.92	0.63
Uri	785	2.1	37	84.19	4.18	0.92	0.63
Younbaek	788	1.7	35	83.14	4.39	0.91	0.63
Imported wheat flour[a]							
SW	728	2.1	36	88.82	3.64	0.93	0.64
ASW	813	1.6	34	86.09	4.4	0.94	0.67
HRW	917	1.2	33	82.27	6.6	0.9	0.65
DNS	997	0.8	30	81.31	7.19	0.91	0.64
Commercial flour[b]							
Com1	1015	0.9	31	83.66	7.45	0.92	0.66
Com2	810	1.4	34	87.38	4.26	0.93	0.68
Com3	837	1.5	34	84.88	4.52	0.93	0.68
LSDc	33	0.1	–	0.31	0.17	0.01	0.01

[a]W soft white, ASW Australian standard white, HRW hard red winter, DNS dark northern spring

[b]Com1 commercial flours for bread baking, Com2 and 3 commercial flour for wet and dry noodles, respectively

[c]Least significant difference (p = 0.05). Differences between two means exceeding this value are significant

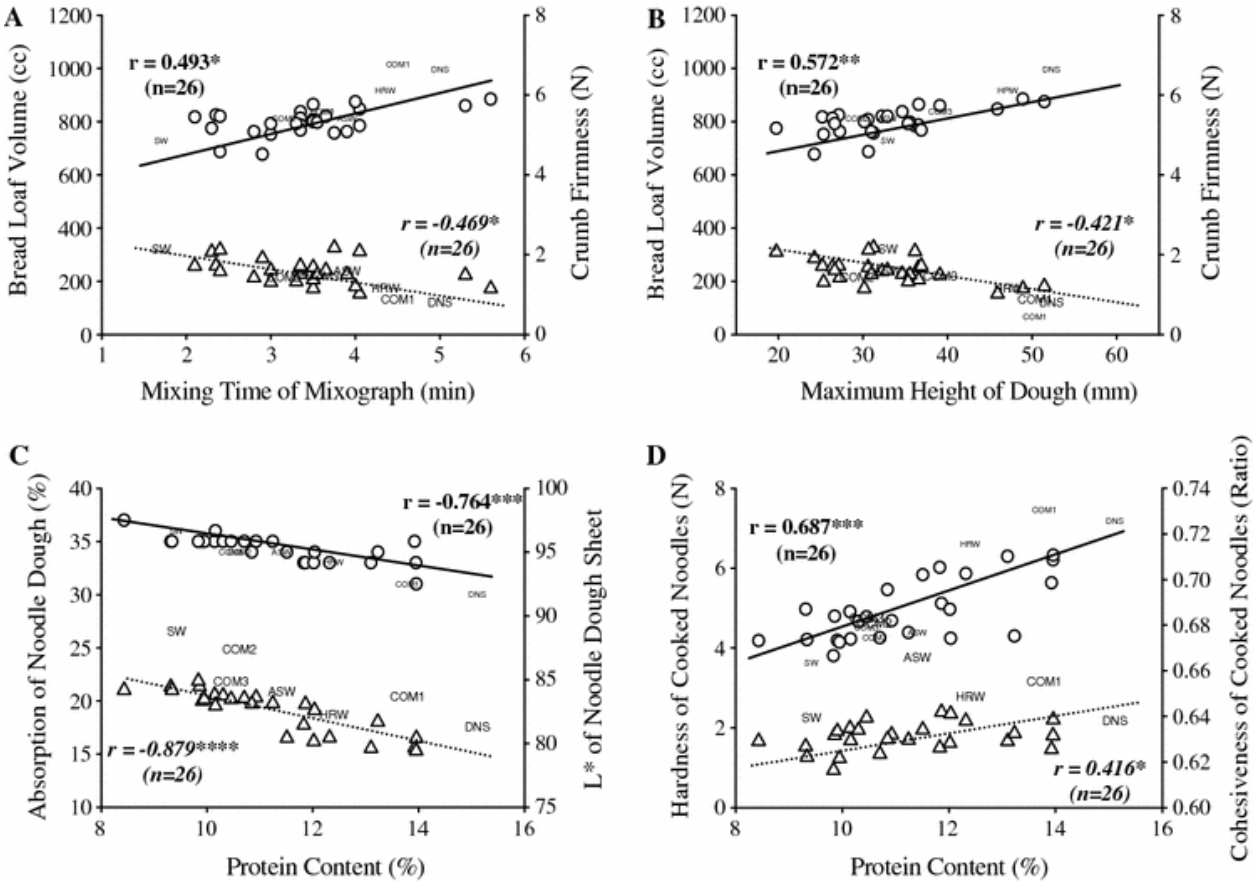

Fig. 2. The relationships between bread quality properties, including bread loaf volume and crumb firmness, and mixing time of mixograph (a), maximum height during dough development (b), and between protein content of flour, noodle dough properties, including optimum water absorption of noodle dough and L* of noodle dough sheet (c), and texture of cooked white salted noodles prepared from 26 Korean wheat flours, hardness and springiness of cooked noodles (d). Open circles and solid lines indicate relationship between bottom and left parameter and open triangles and dotted lines indicate relationship between bottom and right parameter.

In the process of making white salted noodles using Korean cultivars, optimum water absorption of noodle dough and lightness of noodle dough sheet increased from 31 to 37% and from 79.4 to 84.9%, respectively, as protein content decreased. Those noodle making properties were negatively correlated with protein content of Korean wheat flours (Fig. 2c). They were also negatively correlated with optimum water absorption of mixograph, because water absorption of mixograph was influenced by protein content. There was no significant relationship between noodle dough properties and other properties of mixograph and rheofermentometer.

In the texture of cooked white salted noodles from Korean wheat cultivars, hardness ranged from 3.81 to 6.33 N, which was higher than that of SW and lower than that of HRW, DNS and Com1. Hardness of cooked noodles prepared from Baekjoong, Chungkye, Hanbaek, Jinpoom, Olgeuru, Seodun, Sukang, Uri and Younbaek was comparable to that of noodles prepared from Com2 and ASW. Hardness of Namhae and Saeol was comparable to that of Com3. Hardness was positively correlated with protein content ($r = 0.687$, $p < 0.001$, Fig. 2d) and optimum water absorption of mixograph ($r = 0.769$, $p < 0.001$). Springiness of cooked noodles ranged from 0.91 to 0.95 in Korean wheat flours, from 0.90 to 0.94 in imported wheat flours, and from 0.92 to 0.93 in commercial flours. Springiness of cooked noodles from Hanbaek and Sukang was comparable to that of ASW showing higher value than that of imported and commercial flours. Only springiness was positively correlated with SDS-sedimentation volume in Korean wheat cultivars ($r = 0.450$, $p < 0.05$). Cohesiveness of cooked noodles ranged from 0.62 to 0.64 in Korean wheat flours, which was lower than that of ASW and commercial flours (>0.66). Eunpa, Hanbaek, Jeokjoong, Jopoom and Keumkang showed higher cohesiveness than other Korean cultivars, which were comparable to cohesiveness of cooked noodles from SW and HRW. Cohesiveness was positively correlated with protein content ($r = 0.416$, $p < 0.05$, Fig. 2d) and optimum water absorption of mixograph ($r = 0.608$, $p < 0.001$) in Korean wheat cultivars.

2.2.3.3. Effects of glutenin alleles on quality properties

Allelic composition of HMW-GS and LMW-GS in 26 Korean wheat cultivars is shown in Table 3 and Fig. 3. Table 3 shows also pedigree of Korean wheat cultivars, which germplasms from Japan and international maize and wheat improvement centre (CIMMYT) has mainly been introduced to improve early maturation and increasing grain yield in Korean wheats. Korean wheat cultivars were divided into 9 different groups on the basis of HMW-GS composition. Three alleles were identified at each *Glu-1* loci. Null allele was mostly found in *Glu-A1* locus,

7 + 8 subunits and 2.2 + 12 subunits were predominantly found in *Glu-B1* and *Glu-D1* locus, respectively. In LMW-GS composition, Korean cultivars had 14 different groups and three alleles were identified at *Glu-A3*, four at *Glu-B3* locus and three at *Glu-D3* locus. Glu-D3a (65.4%) was the most common allele followed by Glu-B3d (61.5%) and Glu-A3d (50.0%) in Korean wheat cultivars.

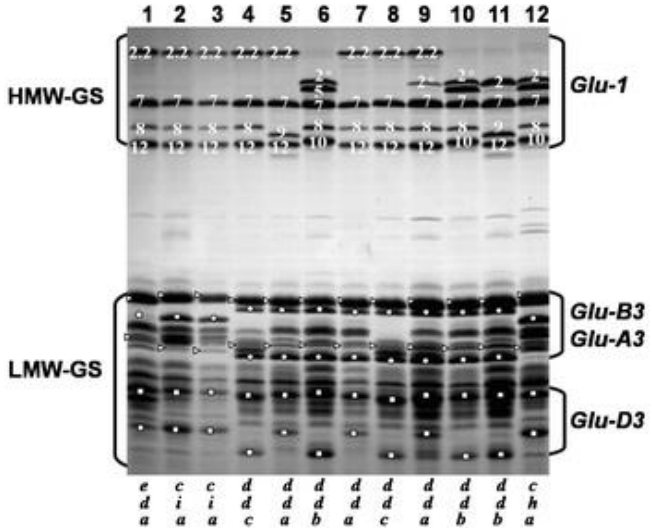

Fig. 3. One-dimensional SDS-PAGE patterns of reduced and alkylated glutenin subunits for allelic variations of HMW-GS and LMW-GS in Korean wheat cultivars. Open circles, open triangles and open squares indicate identified alleles of *Glu-A3 Glu-B3* and *Glu-D3* of LMW-GS, respectively. 1 Ol, 2 Dahong, 3 Geuru, 4 Chungkye, 5 Eunpa, 6 Tapdong, 7 Namhae, 8 Uri, 9 Olgeuru, 10 Alchan, 11 Gobun, 12 Keumkang

Table 3. Allelic compositions of high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), pedigree and usage of Korean wheat cultivars

Cultivar	HMW-GSa			LMW-GSb			Pedigree	Usage
	Glu-A1	Glu-B1	Glu-D1	Glu-A3	Glu-B3	Glu-D3		
Alchan	2*	7 + 8	5 + 10	d	d	b	Suwon210/Tapdong	Noodles
Anbaek	N	7 + 9	2 + 12	e	i	a	Sae/Geuru	Noodles
Baekjoong	2*	13 + 16	2.2 + 12	c	d	a	Keumkang/Olgeuru	Noodles
Chungkye	N	7 + 8	2.2 + 12	d	d	c	Norin4/Sharbatisonora	Noodles
Dahong	N	7 + 8	2.2 + 12	c	i	a	Norin72/Wonkwang	Noodles
Eunpa	N	7 + 9	2.2 + 12	d	d	a	Chukoku81/3/Tob-CNO//Yuksung3/Suwon185	Noodles
Geuru	N	7 + 8	2.2 + 12	c	i	a	Strampelli/69D-3607//Chokwang	Noodles
Gobun	N	7 + 9	2 + 12	d	d	b	Eunpa/Tapdong//Eunpa/Shannung 6521	Noodles
Hanbaek	2*	7 + 8	5 + 10	c	h	c	Shann7859/Keumkang//Guamuehill	Noodles
Jeokjoong	2*	13 + 16	2.2 + 12	c	d	a	Keumkang/Tapdong	Noodles
Jinpoom	N	7 + 8	2.2 + 12	d	d	c	Geuru/Genaro81	Noodles
Joeun	N	13 + 16	2.2 + 12	e	h	a	Eunpa/Suwon242	Noodles
Jokyung	1	7 + 8	5 + 10	c	h	a	Seri82/Keumkang	Noodles/ Bread
Jonong	1	7 + 8	5 + 10	c	h	a	Suwon234/SW80199	Noodles
Jopoom	N	13 + 16	2.2 + 12	d	i	a	Kanto75//OR8500494P/Bezostaya	Noodles
Keumkang	2*	7 + 8	5 + 10	c	h	a	Geuru/Kanto75//Eunpa	Noodles/ Bread
Milsung	N	7 + 8	2.2 + 12	c	d	a	Sirogane//Norin43/Sonalika	Noodles
Namhae	N	7 + 8	2.2 + 12	d	d	a	Ol/Calidad	Noodles
Ol	Nc	7 + 8	2.2 + 12	e	d	a	Norin72/Norin12	Noodles
Olgeuru	2*	7 + 8	2.2 + 12	d	d	a	Geuru/Chokwang//Nishigai143	Noodles
Saeol	N	7 + 8	2.2 + 12	d	d	b	Sirogane//Norin43/Sonalika	Noodles
Seodun	N	7 + 8	2.2 + 12	d	d	c	Geuru/Genaro81	Noodles
Sukang	2*	13 + 16	2 + 12	d	b	a	Suwon266/Asakaje	Noodles
Tapdong	2*	7 + 8	5 + 10	d	d	b	Chukoku81//Suwon158/Toropi	Noodles/ Bread
Uri	N	7 + 8	2.2 + 12	d	d	c	Geuru/Ol	Noodles/ Cookie
Younbaek	2*	13 + 16	2.2 + 12	c	d	a	Keumkang/Tapdong	Noodles

[a]Nomenclature according to Payne and Lawrence (1983)

[b]Nomenclature according to Gupta and Shepherd (1990)

[c]Null allele

Differences in physicochemical and rheological properties of wheat flours and end-use quality of Korean wheat cultivars according to the allelic variations at *Glu-1* and *Glu-3* loci are summarized in Tables 4 and 5. At *Glu-A1* locus, 2* subunit showed higher SDS-sedimentation volume and longer mixing time than cultivars with null allele. Subunits 1 and 2* had coarser particle size of flour (>81.2 μm), higher maximum height of dough (>37.5 mm) and bread loaf volume (819 ml) than null allele (73.3 μm, 28.8 mm and 778 ml, respectively). Cultivars with 7 + 8 subunits had finer particle size of flour (72.4 μm) than other subunits at *Glu-B1* loci (>89.0 μm). These cultivars also showed higher lightness of noodle dough sheet (83.13) than cultivars with 7 + 9 subunits (80.35). Cultivars with 13 + 16 subunits showed higher protein content (12.2%) and optimum water absorption of mixograph (60.3%) than cultivars with 7 + 8 subunits (10.7 and 58.0%, respectively). Cultivars with 5 + 10 subunits showed longer mixing time, higher maximum height during dough development and bread loaf volume than cultivars with 2.2 + 12 and 2 + 12 subunits. Korean wheat cultivar with 2.2 + 12 subunits had finer particle size of flour (74.6 μm), lower SDS-sedimentation volume (36.9 ml), mixing tolerance (14.1 mm) and higher crumb firmness of bread (1.7 N) than cultivars with 5 + 10 subunits (84.3 μm, 47.7 ml, 19.4 mm and 1.3 N, respectively).

Table 4. Differences in physicochemical and rheological properties of wheat flours and characteristics of bread and white salted noodles prepared from Korean wheat cultivars according to the allelic compositions of high-molecular-weight glutenin subunits (HMW-GS) using pair-wise *t*-test

Allele parameter[a]	*Glu-A1*			*Glu-B1*			*Glu-D1*		
	Null	1	2*	7 + 8	7 + 9	13 + 16	5 + 10	2 + 12	2.2 + 12
No. of cultivar	15	2	9	17	3	6	6	3	17
PSI	73.3b	81.2a	87.1a	72.4b	89.0a	91.5a	84.3a	90.7a	74.6b
Amylose	25.5a	24.9a	25.3a	25.5a	25.0a	25.0a	25.3a	24.8a	25.5a
Protein	11.1ab	12.0a	11.1a	10.7b	12.0ab	12.2a	11.5a	12.3a	10.9a
SDSS	37.0b	42.5ab	47.1a	38.0a	46.8a	46.2a	47.7ab	50.5abc	36.9c
Mabs	58.5a	60.00a	58.8a	58.0b	59.8ab	60.3a	59.3a	58.7a	58.5a
Mtime	3.0b	3.8ab	3.9a	3.5a	3.2a	3.3a	4.4a	3.2b	3.1b
Mtol	14.2a	17.3a	16.9a	15.6a	15.8a	14.7a	19.4a	14.8ab	14.1c
Hm	28.8b	44.0a	37.5a	33.1a	31.4a	33.5a	42.5a	33.0b	29.7b
RC	93.6a	94.1a	94.3a	93.9a	93.9a	93.8a	94.5a	94.2a	93.6a
Vol	778b	870a	819a	803a	795a	790a	859a	790b	780b
Firm	1.7a	1.3a	1.5a	1.6a	1.8a	1.5a	1.3c	1.7ab	1.7ab
Nabs	34a	34a	34a	35a	33a	34a	34a	34a	35a
NL	82.43a	81.37a	82.91a	83.13a	80.35b	81.85ab	82.39a	80.76a	82.87a
HD	4.97a	5.88a	4.69a	4.80a	5.56a	5.04a	5.35a	5.04a	4.78a
SP	0.92a	0.93a	0.93a	0.92a	0.92a	0.93a	0.93a	0.93a	0.92a
CO	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a

[a]*PSI* average particle size of flour, *SDSS SDS*-sedimentation volume based on a constant protein weight (300 mg), Mabs water absorption of 10 g mixograph, Mtime mixing time of 10 g mixograph, Mtol mixing tolerance of 10 g mixograph, Hm maximum height during dough development, RC retention coefficient of dough, Vol bread loaf volume, Firm crumb firmness, Nabs optimum water absorption of noodle dough, NL lightness of noodle dough sheet, HD hardness of cooked noodles, SP springiness of cooked noodles, CO cohesiveness of cooked noodles

[b]Values followed by same letters within same locus are not significantly different at $P < 0.05$

Table 5. Differences in physicochemical and rheological properties of wheat flours and characteristics of bread and white salted noodles prepared from Korean wheat cultivars according to the allelic compositions of low-molecular-weight glutenin subunits (LMW-GS) using pair-wise *t*-test

Allele parameter[a]	*Glu-A3*			*Glu-B3*				*Glu-D3*		
	c	d	e	b	d	h	i	a	b	c
No. of cultivar	10	13	3	1	16	5	4	17	4	5
PSI	82.6a	74.6a	83.3a	95.2a	75.0b	88.1ab	77.6b	80.6a	77.3a	73.4a
Amylose	25.3a	25.5a	25.0a	24.9a	25.5a	25.2a	25.2a	25.2a	25.3a	26.0a
Protein	11.3a	10.9a	12.0a	13.2a	10.4b	12.4a	12.4a	11.6a	10.7ab	10.1b
SDSP	37.6a	43.9a	38.8a	52.5a	40.5a	46.0a	33.0a	38.4a	49.9a	42.2a
Mabs	58.4a	58.7a	60.0a	59.0a	58.1a	60.1a	59.5a	59.3a	58.4a	57.1a
Mtime	3.2ab	3.7a	2.6b	3.3abc	3.5ab	3.6abc	2.6c	3.1b	4.4a	3.5ab
Mtol	14.5a	16.6a	13.5a	9.5b	16.1a	18.3a	10.4b	13.8b	20.5a	16.8ab
Hm	35.1a	32.3a	28.7a	35.4a	32.6a	38.7a	26.8b	33.0a	36.6a	29.9a
RC	94.0a	93.9a	93.5a	94.3a	93.9a	94.4a	93.2a	93.9a	94.0a	93.9a
Vol	804a	792a	813a	793ab	786b	840a	803ab	807a	817a	760a
Firm	1.6a	1.6a	1.6a	1.3a	1.7a	1.4a	1.6a	1.6a	1.6a	1.7a
Nabs	35a	34a	34a	34abc	35a	33c	34b	34a	34a	35a
NL	82.71a	82.69a	81.13a	81.69ab	83.19a	81.57ab	81.18b	82.20a	82.52a	83.60a
HD	4.88a	4.90a	5.36a	4.30ab	4.70b	5.47a	5.43ab	5.06a	5.37a	4.21b
SP	0.92a	0.93a	0.92a	0.95a	0.92b	0.93b	0.92b	0.92a	0.92a	0.93a
CO	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a	0.63a

Mixing time of mixograph from cultivars with *Glu-A3d* (3.7 min) was longer than that of cultivars with *Glu-A3e* (2.6 min). At Glu-B3 locus, cultivars with *Glu-B3d* and i had finer particle size of flour (<77.6 μm) than wheats with *Glu-B3h* (88.1 μm). *Glu-B3d* showed the lowest protein content (10.4%) than other alleles (>12.4%). *Glu-B3d* had longer mixing time (3.5 min) and higher mixing tolerance (16.1 mm), optimum water absorption of noodle dough (35%) and lightness of noodle dough sheet (83.19) than *Glu-B3i* (2.6 min, 10.4 mm, 34% and 81.18, respectively). *Glu-B3h* had higher mixing tolerance (18.3 mm) and maximum height during dough development (38.7 mm) than *Glu-B3i* (10.4 mm and 26.8 mm, respectively). *Glu-B3h* also had higher loaf volume (840 ml) and hardness of cooked noodles (5.47 N) and lower optimum water absorption of noodle dough (33%) than *Glu-B3d* (786 ml, 4.70 N and 35%, respectively). At *Glu-D3* locus, protein content of *Glu-D3a* (11.6%) was higher than that of *Glu-D3c* (10.1%). *Glu-D3b* showed longer mixing time (4.4 min) and higher mixing tolerance (20.5 mm) than *Glu-D3a* (3.1 min and 13.8 mm, respectively). *Glu-D3c* showed lower hardness of cooked noodles (4.21 N) than *Glu-D3a* and *Glu-D3b* (5.06 and 5.37 N, respectively).

Korean wheat cultivars with 7 + 8 and 2.2 + 12 subunits at *Glu-1* loci (*Glu-A3c*, *GluB3i* and *Glu-D3a*) showed lower SDS-sedimentation volume (Fig. 4a), shorter mixing time of mixograph (Fig. 4b), lower mixing tolerance (Fig. 4c), higher hardness and lower springiness of cooked noodles (Fig. 4e and f) than cultivars with *Glu-A3d*, *GluB3d* and *Glu-D3a-c*. In cultivars with 1 or 2*, 7 + 8, 5 + 10 subunits, *Glu-A3d*, *GluB3d* and *Glu-D3b* showed higher mixing time of mixograph (Fig. 4b) and bread loaf volume (Fig. 4d), and lower springiness of cooked noodles (Fig. 4f) than *Glu-A3c*, *GluB3h* and *Glu-D3a*.

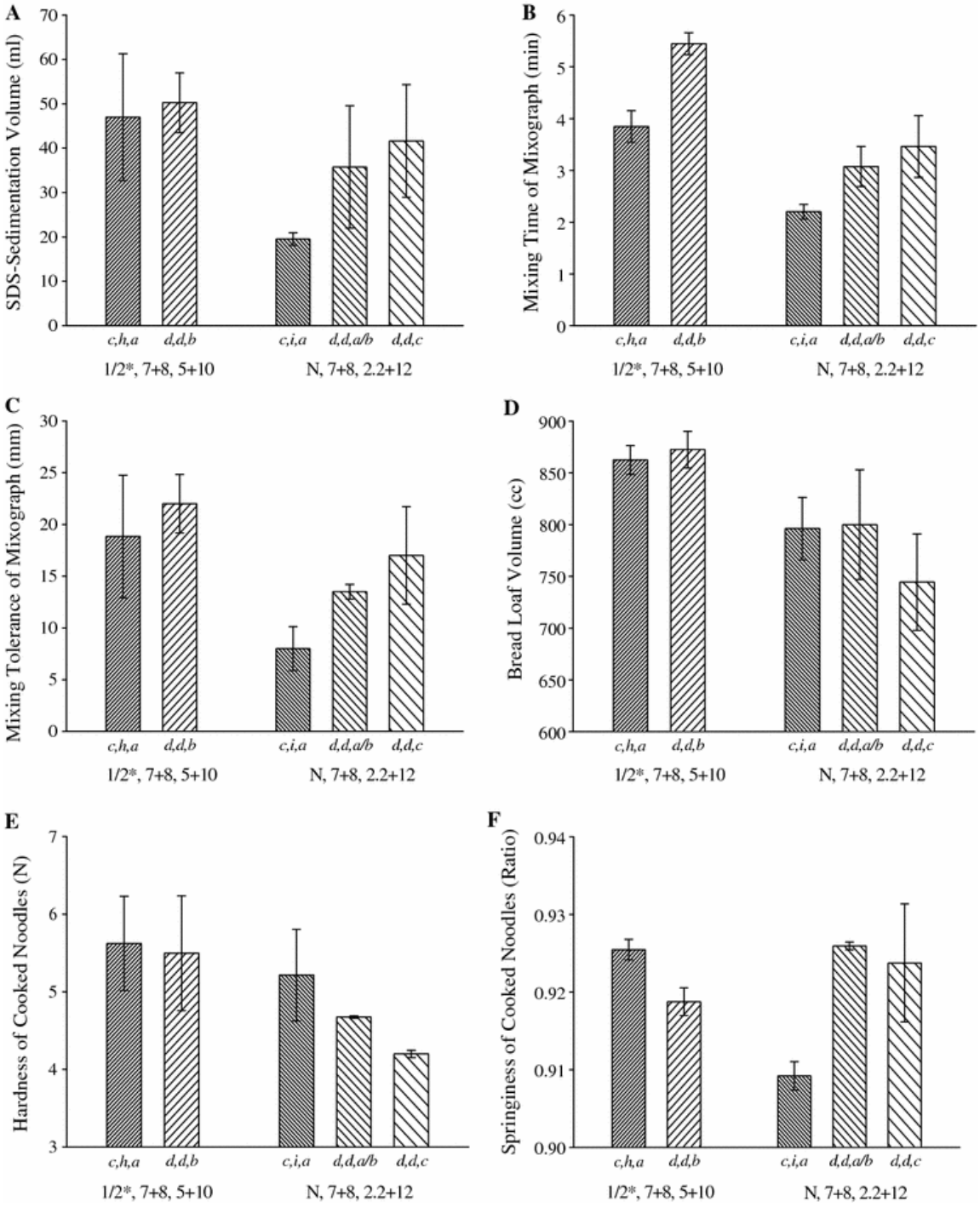

Fig. 4. The difference of SDS-sedimentation volume with constant protein weight (a), mixing time of mixograph (b), mixing tolerance of mixograph (c), bread loaf volume (d), hardness of cooked noodles (e), and springiness of cooked noodles (f) according to the LMW-GS compositions with same HMW-GS compositions of Korean wheat cultivars. Error bars indicate the standard deviation.

2.2.4. Discussion

Although Korean wheat cultivars showed lower loaf volume and harder crumb texture compared to commercial flour for bread, Tapdong showed comparable bread properties to HRW. Tapdong containing 2*, 7 + 8, and 5 + 10 subunits at *Glu-1* loci and Korean wheat cultivars carrying 5 + 10 subunits including Alchan, Hanbaek, Keumkang, Jonong and Jokyung showed higher bread loaf volume than cultivars with 2 + 12 or 2.2 + 12 subunit, which are in agreement with the report of Shewry *et al.* (1992). The higher frequency of null allele at *Glu-A1* locus and 2.2 + 12 subunits at *Glu-D1* locus in Korean wheat cultivars was similar to Japanese wheat cultivars (Payne *et al.* 1983) due to the pedigree of Korean wheats (Table 3). Korean wheats with 2.2 + 12 subunits showed smaller particle size (74.6 μm) than other subunits in *Glu-D1* loci (86.4 μm), which is in agreement with the previous reports (Nakamura *et al.* 1990; Oda *et al.* 1992). Texture of cooked noodles of wheat flours with 2.2 + 12 subunit and around 10% protein content was comparable to that of commercial noodle flours. However, those wheat flours showed higher optimum water absorption of noodle dough and lower lightness of noodle dough sheet than commercial wheat flours. Further evaluations on flour characteristics related to water holding capacity and contributing factors to noodle color such as milling extraction rate, starch damage, brown and yellow pigments and polyphenol oxidase activity should be considered to improve noodle quality. The variation in *Glu-B1* locus affected the lightness of cooked noodles, in which 7 + 8 subunits showed higher lightness of noodle dough sheet than 7 + 9 subunits due to the low protein content of wheat flours (Table 1). Korean wheat cultivars with 1 or 2* subunit showed stronger dough properties and higher bread loaf volume than cultivars with null allele, which were in agreement with Payne *et al*. (1987). Liu *et al.* (2005) reported that *Glu-B1* and *Glu-D1* accounted for SDS sedimentation volume and mixing properties in Chinese bread wheat. Primard *et al.* (1991) reported there was no difference found between 7 + 8 and 7 + 9 subunits in bread-baking quality among the same genetic backgrounds of Great Plains germplasm. No significant differences were found in properties of white salted noodles at *Glu-1* loci.

Korean wheat cultivars showed high frequency of *Glu-A3d*, which this frequency was also found in Japanese and French wheats (Branlard *et al.* 2003; Tanaka *et al*. 2005) but other alleles in *Glu-A3* were predominantly found in Argentinean, Australian, China and US grown wheats (Eagles *et al.* 2002; Lerner *et al.* 2009; Li *et al.* 2009; Shan *et al.* 2007). Korean wheats with *Glu-A3d* had longer mixing time than *Glu-A3e*. Glu-A3b allele was not found in Korean

cultivars. The effects of *Glu-A3* on other mixing and dough properties and bread and noodle qualities were not significantly different. The effect of *Glu-A3b* and *Glu-A3d* alleles on dough strength was stronger than other alleles and negative effect of Glu-A3e on dough rheological characteristics was reported in many previous studies (Branlard *et al.* 2001; Eagles *et al.* 2002; Gupta *et al.* 1989, 1991; Metakovsky *et al.* 1990; Vawser *et al.* 2002).

In the frequency of *Glu-B3* alleles, *Glu-B3d* was the most common allele in Korean wheat cultivars but this allele was rarely found in Argentinean, Australian, French, Japanese and US wheats (Branlard *et al.* 2003; Eagles *et al.* 2002; Lerner *et al.* 2009. Shan *et al.* 2007; Tanaka *et al.* 2005). *Glu-B3d* was the second most frequent allele in both landraces (28.76%) and released varieties (32.44%) in Chinese wheats (Li *et al.* 2009). Korean wheat cultivars carrying *Glu-B3d* and *Glu-B3h* had stronger gluten strength than *Glu-B3i*, which were in agreement with the study of Maucher *et al.* (2009). Cultivars with *Glu-B3h* had higher bread loaf volume than others because they also contain 5 + 10 subunits at the *Glu-D1* locus, which had higher bread loaf volume than cultivars with 2 + 12 or 2.2 + 12 subunits. *Glu-B3d* showed brighter noodle color and softer texture than others because *Glu-B3d* showed the lowest protein content. Peña *et al.* (2004) reported that wheat lines with *Glu-D1d* and *Glu-B3d* showed the strongest gluten strength followed by groups possessing *Glu-D1d* combined with *Glu-B3b*, *Glu-D3f* and *Glu-B3g*. They also reported that *Glu-B3i* and *Glu-B3h* alleles showed intermediate gluten strength. He *et al.* (2005) reported that *Glu-A3d* and *Glu-B3d* were considered to be slightly better than others in dry white Chinese noodle quality.

At the *Glu-D3* locus, the frequency of *Glu-D3a* was higher than other alleles at *Glu-D3* loci. However, this allele was rarely found in Argentinean, Australian, French (Branlard *et al.* 2003; Eagles *et al.* 2002; Lerner *et al.* 2009) and the frequencies of *Glu-D3* alleles were approximately equal in US hard wheats (Shan *et al.* 2007). The relationships between *Glu-D3* and quality properties of Korean wheat cultivars showed that *Glu-D3b* could be suitable to increase dough strength during mixing. Cultivars with *Glu-D3c* could make softer texture of cooked noodles than cultivars with other alleles due to their lower hardness of cooked noodles. *Glu-D3b* allele showed stronger dough strength than *Glu-D3a* and *Glu-D3c* in Australian and New Zealand cultivars (Gupta *et al.* 1989, 1991; Metakovsky *et al.* 1990; Luo *et al.* 2001). However, Branlard et al. (2001) reported that *Glu-D3a* positively affected dough strength in French wheat and Vawser *et al.* (2002) reported that no difference was found in dough strength at *Glu-D3* locus.

Korean wheat cultivars with 2.2 + 12 subunits in *Glu-D1* loci showed shorter mixing time of mixograph and lower bread loaf volume than cultivars with 5 + 10 subunits regardless of the combination with allelic variations of *Glu-3*. Among these cultivars, *Glu-A3d* and *Glu-B3d* showed softer and more elastic texture of cooked noodles than wheat cultivars with 5 + 10 subunits. Branlard et al. (2001) proposed that the effects of *Glu-3* were generally additive to *Glu-1*. Therefore, it should be considered to increase the frequency of *Glu-B3b* in Korean wheat cultivars with 5 + 10 subunits to improve dough strength for better bread-baking quality. Further study is required to examine the contribution of allelic variations at *Glu-3* in noodle making process and texture of cooked noodles prepared from wheat cultivars with 2.2 + 12 subunits for improving noodle quality.

Chapter-3

Influence of puroindoline genotypes on grain characteristics, physico-chemical properties of flour and end-use quality of Korean wheats

Abstract

Puroindoline genotypes, grain characteristics, physico-chemical properties of flour and end-use qualities of Korean wheat cultivars were evaluated to determine the influence of puroindolines genotypes and to provide on those characteristics. Nine Korean wheat cultivars carried *Pina-D1a*/*Pinb-D1a*, fourteen had *Pina-D1a*/*Pinb-D1b,* and three had *Pina-D1b*/*Pinb-D1a*. *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b* genotypes showed significantly higher 1000-kernel weight and hardness index in grain characteristics, higher flour yield, average particle size, ash and protein content, SDS-sedimentation volume, water absorption and mixing time of mixograph, thickness of noodle dough sheet and lower values in lightness of flour and cookie diameter than *Pina-D1a*/*Pinb-D1a* genotype. The *Pina-D1b*/*Pinb-D1a* genotype showed significantly higher damaged starch content and water retention capacity than other genotypes. Hardness index of grain was positively correlated with flour yield and average particle size of flour. Those parameters were positively correlated with damaged starch, water retention capacity, SDS-sedimentation volume and water absorption of mixograph, and negatively correlated with lightness of wheat flour. In end-use qualities, thickness and lightness of noodle dough sheet and cookie diameter were correlated with hardness index, milling performances and physical properties. However, bread volume and texture of cooked noodles were not influenced by milling performances and physical properties.

3.1. Introduction

Grain hardness, either soft or hard, has been often used as a key determinant in differentiating wheat classes because hardness has influences on milling and end-use qualities of wheat (Morris and Rose 1996). Hard wheats generally produce coarser particles of flour and greater number of mechanically damaged starch than soft wheats. Therefore, hard wheat flours are generally used for bread and other yeast-leavened food, whereas soft wheats are preferred for pastries, cakes and cookies.

Symes (1965) reported that grain hardness of wheat is controlled by the hardness locus (*Ha*) located on the short arm of chromosome 5D. Barlow *et al.* (1973) proposed that the major gene must control the production of water-soluble proteins which surround starch granules. Greenwell and Schofield (1986) reported that friabilin, water-soluble 15-kDa protein found in the surface of starch and strongly associated with grain hardness, was identified in soft wheat

starch granules. Jolly *et al.* (1993) reported that friabilin was abundant in all soft wheat starch granules, whereas rare on hard wheat starches and absent in durum wheat.

Two major polypeptides, termed as puroindoline a (*Pina*) and b (*Pinb*), were identified as the major components of friabilin (Blochet *et al.* 1993, Gautier *et al.* 1994, Rahman *et al.* 1994). Genes coding for puroindolines are tightly linked to the *Ha* locus on chromosome 5D and probably function together with *Ha* locus (Sourdille *et al.* 1996, Giroux and Morris 1997, 1998). Soft wheats contain both *Pina-D1a* and *Pinb-D1a* genotypes, which are designated as the wild type of puroindolines. Up to date, 9 allelic variations at *Pina-D1* locus and allelic variations at *Pinb-D1* locus in common wheats were reviewed by Morris and Bhave (2008).

Hard wheats carrying *Pina-D1b* allele showed different effects on milling and end-use qualities from *Pinb-D1a* genotypes. *Pina-D1b* genotypes showed harder grain texture, higher flour yield, protein content, damaged starch, water absorption, color score of noodles and loaf volume of bread and lower lightness and ash content of flour than hard wheats with *Pinb-D1b* (Giroux *et al.* 2000, Martin *et al.* 2001, Nagamine *et al.* 2003, Cane *et al.* 2004, Chen *at al.* 2007). In Korea, wheat breeding has been focused on increasing grain yield and early maturation and about 30 cultivars have been developed with the pedigree method since 1970s. Recently, quality improvement associated with flour and end-use has received more attention by wheat breeders than ever in Korea. However, the information about influences of puroindoline genotypes on milling performance, characteristics of flour and end-use qualities are not available in Korean wheats. This study was conducted to elucidate the relationships among puroindoline genotypes, grain properties and milling performance, as well as to determine the influence of puroindoline genotypes on physico-chemical properties of flour and qualities of bread, white salted noodles, and sugar-snap cookies made from Korean wheat cultivars in order to provide useful information for improving wheat quality in Korean wheat breeding programs.

3.2. Materials and methods

3.2.1. Plant materials

Twenty six leading cultivars of Korean wheats were harvested at Iksan (Upland Crop Experimental Farm of National Institute of Crop Science, RDA) in 2007. The seeds were sawn on October 20 and cultivated following the standard method of wheat in National Institute Crop

Science. Mean temperature was higher (9.7℃) than that of average year (8.7℃) and average precipitation was lower (387.6 mm) than that of average year (492.0 mm).

3.2.2. PCR conditions for puroindoline genotypes

Genomic DNA was extracted from 100 mg of young leaf tissue using the Genomic DNA prep kit (Solgent Co., Korea) according to the manufacturer's instructions. The allelic variations for puroindolines were evaluated by the procedure described by Gautier *et al.* (1994).

3.2.3. Grain characteristics

Test weight and 1000-kernel weight were measured by Grain Scale (Seedburo Equipment Co., USA) and Seed Counter (Pfeuffer GmbH, Germany), respectively. Hardness index was measured by the single kernel characterization system (SKCS) 4100 (Perten Instruments AB, Sweden) according to the Approved Method 55-31 (AACC, 2000).

3.2.4. Milling performances and flour characteristics

Wheat was milled using a Bühler experimental mill according to AACC Approved Methods 26-10. Two kilograms of wheat were conditioned overnight to reach 15% moisture content and then milled with feed rate of 100 g/min and roll settings of 8 and 5 in break rolls and 4 and 2 in reduction rolls. After milling, flour yield was calculated as the proportion of break and reduction flours to total products and break flour yield was calculated by the proportion of break flours to flour yield.

Distribution of flour particle size was measured by the multi-wavelength laser particle size analyzer LS13320 (Beckman Coulter, Inc., USA). Lightness of flour was measured by Minolta CM-2002 (Minolta Camera Co., Japan) with 11 mm measurement aperture. Moisture, ash content and water retention capacity of wheat flour were determined according to Approved Methods 44-15A, 08-01 and 56-11. The determination of starch damage content was carried out following the procedure described by Gibson *et al.* (1992) using an enzymatic assay kits (MegaZyme Pty., Ltd., Australia). The SDS-sedimentation test was performed on a constant protein (300 mg) basis according to the procedure of Baik *et al*. (1994). Optimum water absorption and mixing time of wheat flour were determined using 10g mixograph (National Mfg., USA) according to the approved method, 54-40A (AACC 2000). Dough development was measured by rheofermentometer (Chopin, France) according to the procedure of

Czuchajowska and Pomeranz (1993).

3.2.5. Bread baking

Bread was baked according to the straight-dough methods described by Finney (1984). The ingredients of baking formula consisted of 100 g (14% moisture basis) of flour, 6 g of sugar, 3 g of shortening, 1.5 g of salt, 5.0 g of fresh yeast, 50 mg of ascorbic acid, and 0.25 g of barley malt (about 50 DU/g, 20℃). The dough was fermented in a cabinet at 30℃ and 85% relative humidity for 70 minutes with two punches and a proof period of 60 min, and then baked at 210℃ for 18 min. Bread loaf volume was measured by rapeseed displacement in a graduated chamber after cooling for two hours at the room temperature.

3.2.6. Preparation of white salted noodles

White salted noodles were prepared with 34% water absorption percent of noodle dough according to the methods described by Park *et al*. (2002). The procedure of noodle making was as follows: 100 g of flour (14.0% moisture basis) was mixed to 34% absorption with 6% sodium chloride solution using a pin mixer (National Mfg., USA) for 4 min with the head speed of 86 rpm. Dough was passed through the rollers of a noodle machine (Ohtake Noodle Machine Mfg., Japan) at 8 rpm and 3 mm gap; dough was folded and put through the sheeting rollers. The folding and sheeting were repeated twice. The dough sheet was rested for 1 hr and then put through the sheeting rollers three times at progressively decreasing gaps of 2.40, 1.85 and 1.30 mm. A piece of noodle sheet was placed in plastic bags for determination of color. Lightness of the dough sheet was measured by Minolta CM-2002 (Minolta Camera Co., Japan) with 11 mm measurement aperture. The rest of the dough sheet was cut through no. 12 cutting rolls into strips about 30 cm in length. The rest of the dough sheet was cut through No. 12 cutting rollers into noodle strands of about 30 cm in length, with cross section of 0.3 × 0.2 cm.

3.2.7. Textural properties of cooked noodles

Raw noodles (20 g) were cooked at the determined cooking time in 500 ml of boiling distilled water for 18 min and then rinsed with cold water. Two replicates of cooked noodles were evaluated by texture profile analysis (TPA) using TA-XT2 Texture Analyser (Stable Micro Systems, England) within 5 min after cooking. One set of five strands of cooked noodles was placed parallel on a flat metal plate and compressed crosswise twice to 70% of their original

height using a 3.175 mm metal blade at the speed of 1.0 mm/sec. From force-time curves of the TPA, hardness, springiness and cohesiveness were determined according to the description of Park *et al*. (2002).

3.2.8. Cookie baking

The cookie formula and baking procedures were followed as described by Finney *et al.* (1950) in micro method III. Baked cookies were rested for two hours at the room temperature and their diameter was measured.

3.2.9. Statistical analysis

Statistical analysis of data was performed by SAS software (SAS Institute, NC, USA) using Fisher's least significant difference procedure (LSD), analysis of variance (ANOVA) and Pearson correlation coefficient. Differences were considered to be significant at $P < 0.05$, unless otherwise specified. All data were determined at least in duplicate and all were averaged.

3.3. Results

3.3.1. Grain characteristics and milling performances

Table 1 shows pedigree, growth habit, heading date, maturing date and yield of 26 Korean wheat cultivars. Based on the pedigree of Korean wheat cultivars, Germplasm from Japan and International Maize and Wheat Improvement Centre (CIMMYT) has mainly contributed to early maturation and increasing grain yield. Korean wheat cultivars developed in 2000s earlier heading and maturation date and higher grain yield than cultivars developed in 1980s and 1990s although there was no differences in growth habit and culm length. Nine Korean wheat cultivars (Chungkye, Dahong, Jonong, Milseong, Namhae, Ol, Olgeuru, Saeol, Uri and Younbaek) showed *Pina-D1a/Pinb-D1a* genotype (Table 2).

Table 1. Released year, pedigree, growth habit, heading date, maturing date and yield of 26 Korean wheat cultivars

Cultivar	Released Year	Pedigree	Vernalization (I-VII)	Heading date (M. D.)	Maturing date (M. D)	Culm length (Cm)	Yield (MT/ha)
Ol	1976	Norin72/Norin12	III	5. 5	6. 13	85	3.84
Dahong	1979	Norin72/Wonkwang	III	5. 2	6. 9	75	3.93
Chungkye	1979	Norin4/Sharbatisonora	III	4. 28	6. 8	74	4.46
Geuru	1980	Strampelli/69D-3607//Chokwang	IV	4. 29	6. 8	71	4.51
Eunpa	1982	Chukoku81/3/Tob-CNO//Yuksung3/Suwon185	III	5. 1	6. 11	76	3.95
Tapdong	1986	Chukoku81//Suwon158/Toropi	IV	5. 3	6. 11	62	4.70
Namhae	1988	Ol/Calidad	III	5. 2	6. 11	81	5.41
Uri	1992	Geuru/Ol	III	4. 27	6. 7	79	5.29
Olgeuru	1994	Geuru/Chokwang//Nishigai143	III	4. 26	6. 8	80	4.77
Alchan	1995	Suwon210/Tapdong	III	4. 30	6. 11	68	4.89
Gobun	1996	Eunpa/Tapdong//Eunpa/Shannung6521	III	4. 30	6. 11	72	4.78
Keumkang	1997	Geuru/Kanto75//Eunpa	III	4. 28	6. 9	73	4.22
Seodun	1997	Geuru/Genaro81	III	4. 29	6. 9	71	4.85
Saeol	1997	Sirogane//Norin43/Sonalika	II	4. 27	6. 8	66	5.32
Jinpoom	1998	Geuru/Genaro81	III	5. 1	6. 11	75	5.30
Milseong	1998	Sirogane//Norin43/Sonalika	II	4. 27	5. 28	70	5.24
Joeun	2000	Eunpa/Suwon242	III	4. 21	5. 29	74	4.14
Anbaek	2001	Sae/Geuru	IV	4. 30	6. 8	70	5.42
Jopoom	2001	Kanto75//OR8500494P/Bezostaya	III	4. 20	6. 1	64	4.54
Jonong	2003	Suwon234/SW80199	III	4. 17	5. 30	67	4.65
Jokyung	2004	Seri82/Keumkang	II	4. 19	6. 2	81	5.19
Younbaek	2005	Keumkang/Tapdong	III	4. 23	6. 4	77	6.05
Baekjoong	2007	Keumkang/Olgeuru	II	4. 29	6. 7	77	5.35
Jeokjoong	2007	Keumkang/Tapdong	II	4. 28	6. 7	78	5.33
Sukang	2008	Suwon266/Asakaze	III	4. 28	6. 8	90	4.72
Hanbaek	2008	Shann7859/Keumkang//Guamuehill	IV	4. 27	6. 8	89	5.05

Table 2. Classification of Korean wheat cultivars with respect to the puroindolines genotypes

Genotype[a]	No. of line	Korean wheat cultivar
Pina-D1a *Pinb-D1a*	9	Chungkye, Dahong, Jonong, Milseoung, Namhae, Ol, Olgeuru, Saeol, Uri,
Pina-D1a *Pinb-D1b*	14	Alchan, Anbaek, Baekjoong, Eunpa, Geuru, Gobun, Hanbaek, Jeokjoong, Jipoom, Jokyung, Jopoom, Keumkang, Seodun, Tapdong,
Pina-D1b *Pinb-D1a*	3	Joeun, Sukang, Younbaek

Fourteen cultivars had *Pina-D1a/Pina-D1b* allele and three cultivars (Joeun, Sukang and Younbaek) carried *Pina-D1b/Pinb-D1a* allele. *Pina-D1a* allele was 447bp in agarose gel electrophoresis of PCR, but no product was found in *Pina-D1b* (Fig. 1-A) *Pinb-D1a* and *Pinb-D1b* showed 320bp and 200bp, respectively after restriction with *BsrB*I of PCR products (447bp) of *Pinb-D1* allele (Fig. 1-B).

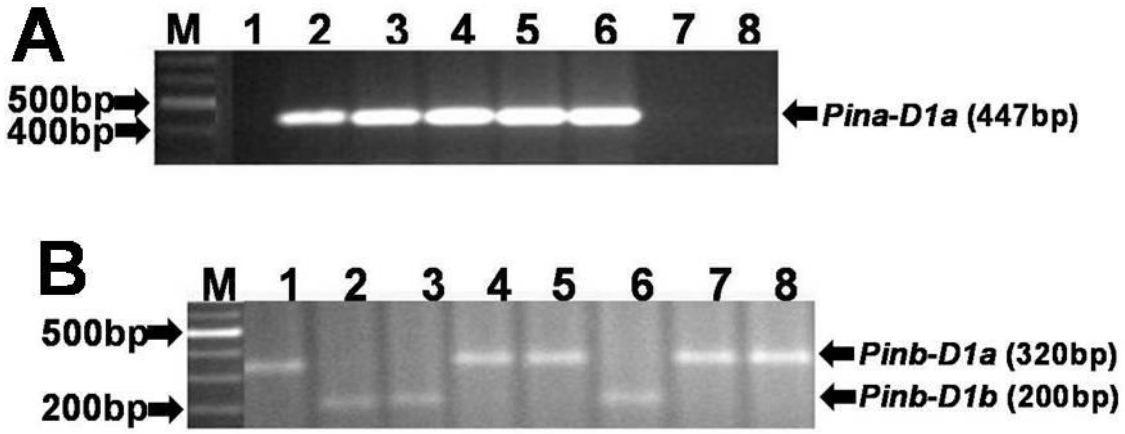

Fig. 1. Agarose gel electrophoresis of PCR amplified Pina-D1 allele (A) and Pinb-D1 allele (B) cut with BsBrI of Korean wheat cultivars. M, molecular size marker; 1, Jokyung; 2, Geuru; 3, Keumkang; 4, Uri; 5, Jonong; 6, Tapdong; 7, Joeun; 8, Younbaek.

Table 3 summarizes grain characteristics, milling performances, physico-chemical properties of wheat flour, dough rheology and end-use quality of Korean wheat flours with different puroindoline genotypes. Korean wheats carrying *Pina-D1a*/*Pinb-D1a* showed lower thousand kernel weight, hardness index of grain and flour yield (38.83 g, 31.73 and 62.20%, respectively) than wheats with *Pina-D1a*/*Pinb-D1b* (43.82 g, 64.91 and 69.54%, respectively) and *Pina-D1b*/*Pinb-D1a* (39.06 g, 67.50 and 71.07%, respectively).

Table 3. Differences in grain characteristics, milling performances, physico-chemical properties of flour, dough rheology and end-use quality in Korean wheat cultivars with all different puroindoline genotypes using pair-wise *t*-test

Characteristics	Allele		
	Pina-D1a *Pinb-D1a*	*Pina-D1a* *Pinb-D1b*	*Pina-D1b* *Pinb-D1a*
Number of cultivar	9	14	3
Grain characteristics			
Test weight (g)	811.67a[a]	822.57a	829.50a
1000-kernel weight (g)	38.83b	43.82a	39.06ab
Hardness index	31.73b	64.91a	67.50a
Milling performances			
Flour yield (%)	62.20b	69.54a	71.07a
Proportion of break flour (%)	29.40a	26.99a	27.98a
Physico-chemical Properties of flour			
Average particle size (μm)	64.26b	86.72a	96.62a
Ash (%)	0.46b	0.51a	0.57a
Damaged starch (%)	2.08c	3.50b	3.81a
Water retention capacity (%)	65.18c	70.41b	73.95a
Lightness of flour	90.73a	89.22b	88.99b
Protein (%)	10.36b	11.39ab	12.81a
Amylose (%)	25.42a	25.03a	24.59a
SDS-sedimentation volume (ml)	29.72b	47.14a	45.17a
Dough Rheology			
Absorption of mixograph (%)	57.33b	59.43a	59.67a
Mixing time of mixograph (min)	2.99b	3.70a	3.22a
Maximum height of dough (mm)	29.93a	35.00a	32.85a
End-use qualities			
Loaf volume (ml)	793.89a	801.43a	790.83a
Thickness of noodle dough sheet (mm)	1.77b	1.85a	1.91a
Lightness of noodle dough sheet	83.47a	82.14a	81.42a
Hardness of cooked noodles (N)	4.64a	5.13a	4.96a
Springiness of cooked noodles (Ratio)	0.92a	0.93a	0.93a
Cohesiveness of cooked noodles (Ratio)	0.63a	0.63a	0.63a
Cookie diameter (mm)	80.84a	76.56b	76.64b

[a]Values followed by same letters within same characteristic are not significantly different at $p < 0.05$

However, there were no significant differences in grain characteristics and milling performances between *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b* genotypes. No significant differences in test weight and the proportion of break flour were found among puroindoline genotypes. In Korean wheat cultivars with all different puroindoline genotypes, flour yield was positively correlated with 1000 kernel weight ($r = 0.474$, $P < 0.05$) and hardness index ($r = 0.597$, $P < 0.001$, Fig. 2-A). Hardness index of grain was positively correlated with test weight ($r = 0.443$, $P < 0.05$) and negatively correlated with the proportion of break flour ($r = -0.444$, $P < 0.05$). In Korean wheats with *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b*, flour yield was negatively correlated with test weight ($r = -0.528$, $P < 0.05$, Fig. 3-A) and positively correlated with the proportion of break flour ($r = 0.575$, $P < 0.05$, Fig. 3-B).

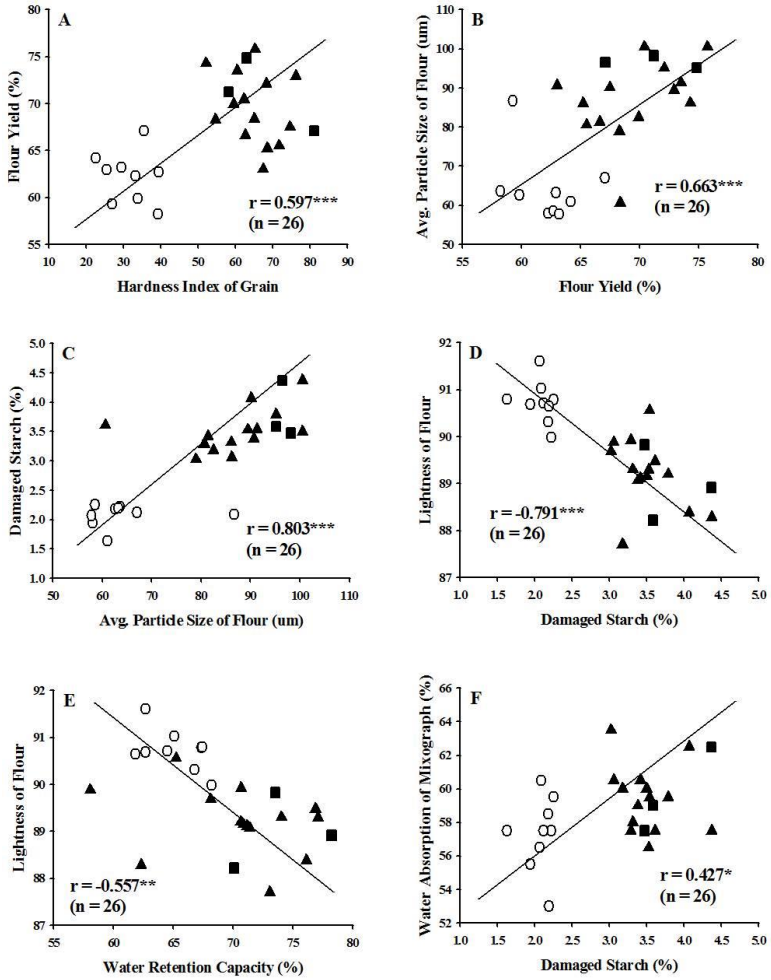

Fig. 2. The relationships between grain characteristics and physical properties of Korean wheat flours with all different puroindoline genotypes. A, hardness index of grain and flour yield; B, flour yield and average of particle size of flour; C, average particle size of flour and damaged starch; D, damaged starch and water retention capacity; E, damaged starch and lightness of flour; F, water retention capacity and lightness of flour. ○; Korean wheats with *Pina-D1a/Pinb-D1a*, ▲; Korean wheats with *Pina-D1a /Pinb-D1b*, ■; Korean wheats with *Pina-D1b/Pinb-D1a*.

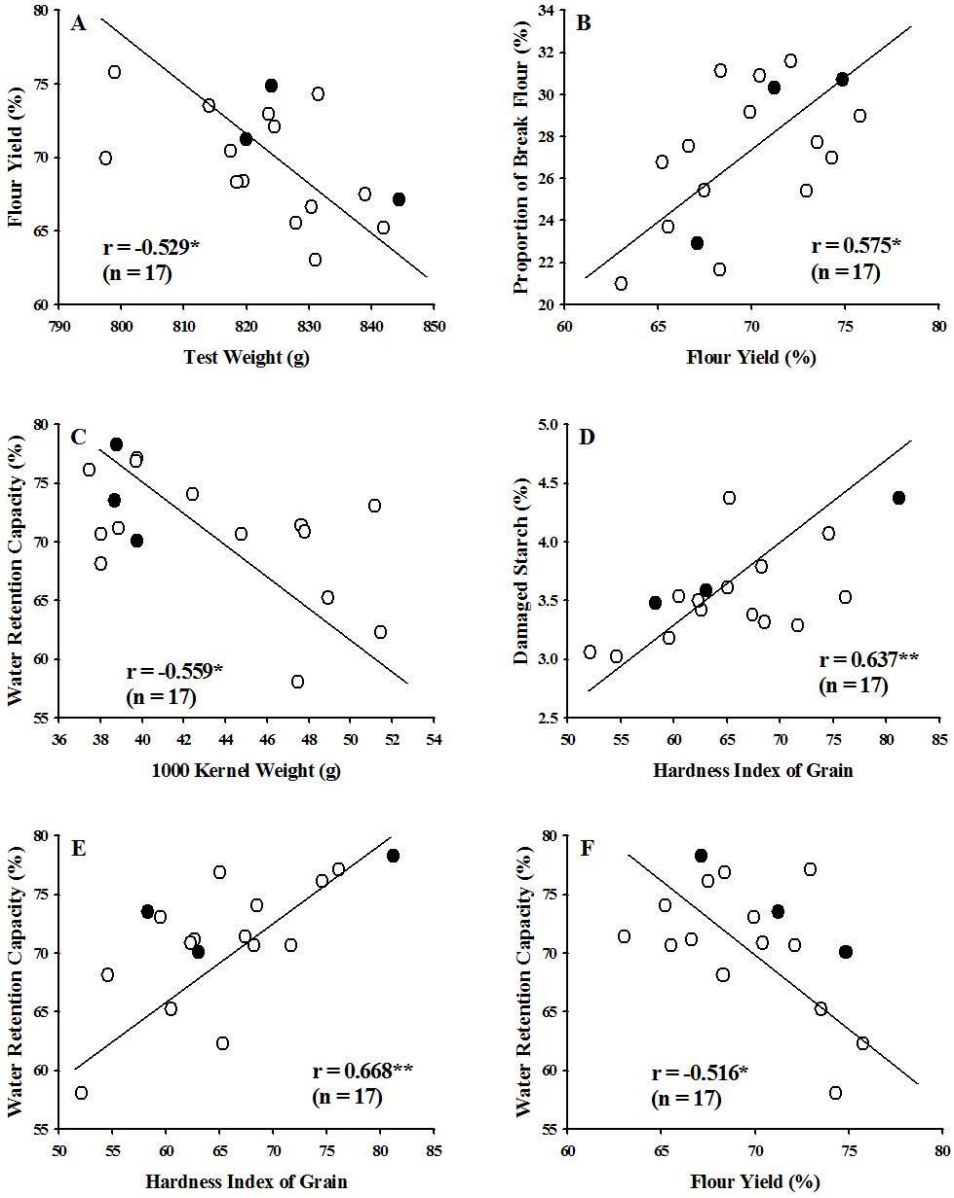

Fig. 3. The relationships between grain characteristics and physical properties of Korean wheat flours with *Pina-D1a/Pinb-D1b* and *Pina-D1b/Pinb-D1a.* A, test weight and flour yield; B, 1000 kernel weight and water retention capacity; C, hardness index and damaged starch; D, hardness index and water retention capacity; E, flour yield and proportion of break flour; F, flour yield and water retention capacity. ○; Korean wheats with *Pina-D1a/Pina-D1b*, ●; Korean wheats with *Pinb-D1a /Pinb-D1a*.

3.3.2. Physico-chemical properties and dough rheology

In Korean wheats with *Pina-D1a*/*Pina-D1b* allele, ash and protein content, SDS-sedimentation volume, optimum water absorption and mixing time of mixograph were higher than those of *Pina-D1a*/*Pinb-D1a* while average particle size of wheat flour and lightness of flour were lower than those of *Pina-D1a*/*Pinb-D1a* (Table 3). But there were no significant differences in these properties between *Pina-D1b/Pinb-D1a* and *Pina-D1a/Pinb-D1b*. Damaged starch and water retention capacity of *Pina-D1b*/*Pinb-D1a* were higher than those of other genotypes and *Pina-D1a*/*Pinb-D1b* showed higher values in these properties than *Pina-D1a*/*Pinb-D1a.* There were no significant differences in amylose content and maximum height during dough development among puroindoline genotypes.

In Korean wheat cultivars with all different puroindoline genotypes, average particle size of flour was positively correlated with flour yield (r = 0.663, $p < 0.001$, Fig. 2-B) and damaged starch content (r = 0.803, $p < 0.001$, Fig. 2-C). Average particle size of flour was also positively correlated with ash and protein content, SDS-sedimentation volume, optimum water absorption of mixograph and negatively correlated with lightness of wheat flour. Damaged starch content was positively correlated with hardness index of grain, flour yield, and water retention capacity. Lightness of wheat flour was negatively correlated with damaged starch (r = -0.791 $P < 0.001$, Fig. 2-D) and water retention capacity (r = -0.557, $P < 0.05$, Fig. 2-E). SDS-sedimentation volume was positively correlated with mixing time of mixograph and maximum height during dough development. Optimum water absorption of mixograph was negatively correlated with hardness index of grain and the proportion of break flour and positively correlated with average particle size of flour, and protein and damaged starch content (r = 0.427, $p < 0.05$, Fig. 2-F). There were no significant relationships between mixing time of mixograph and maximum height, grain characteritcs, milling performances and physico-chemical properties of wheat flour.

In Korean wheat cultivars with *Pina-D1a/Pinb-D1b* and *Pina-D1b/Pinb-D1a*, hardness index was positively correlated with damaged starch (r = 0.637, $P < 0.01$, Fig. 3-C) and water retention capacity (r = 0.668, $P < 0.01$, Fig. 3-D) and water retention capacity was negatively correlated with 1000 kernel weight (r = -0.559 $P < 0.05$, Fig. 3-E), flour yield (r = -0.516 $P < 0.05$, Fig. 3-F) and maximum height during dough development. There was no significant correlation between rheological properties, grain characteristics, milling performances and

physico-chemical properties, although maximum height during dough development was positively correlated with SDS-sedimentation volume and mixing time of mixograph.

3.3.3. End-use qualities

Pina-D1a/Pinb-D1a showed lower thickness of noodle dough sheet and larger cookie diameter than *Pina-D1a/Pinb-D1b* and *Pina-D1b/Pinb-D1a*. There were no significant differences in these properties between *Pina-D1b/Pinb-D1a* and *Pina-D1a/Pinb-D1b*. No significant differences were found in loaf volume of bread, lightness of noodle dough sheet and texture of cooked noodles among puroindoline genotypes.

Table 4 shows the relationships among grain characteristics, milling performances, physic-chemical properties and end-use qualities of Korean wheat cultivars with all different puroindoline genotypes. Loaf volume of bread was positively correlated with mixograph properties and maximum height of dough. Thickness of noodle dough sheet was positively correlated with hardness index, flour yield, average particle size, damaged starch and protein content, and optimum water absorption of mixograph in all different puroindoline genotypes. Those properties were negatively correlated with lightness of noodle dough sheet and cookie diameter. Flour yield was positively correlated with thickness of noodle dough sheet and negatively correlated with cookie diameter. The proportion of break flour was positively correlated with lightness of noodle dough sheet and cookie diameter. Protein content and optimum water absorption of mixograph were positively correlated with hardness and cohesiveness of cooked noodles. The proportion of break flour and average particle size were also correlated with those parameters of cooked noodles.

Table 4. Correlation coefficients among grain characteristics, milling performances, physico-chemical properties and end-use qualities of Korean wheat cultivars with all different puroindoline genotypes

Parameters	*End-use quality*						
	Bread loaf volume	White salted noodles					Cookie diameter
		Dough sheet		Texture of cooked noodles			
		Thickness	Lightness	Hardness	Springiness	Cohesiveness	
Grain characteristics							
Test weight	-0.038	0.532** [a]	-0.381	0.360	-0.144	-0.005	-0.298
1000-kernel weight	0.194	0.199	-0.331	0.120	0.169	0.378	-0.330
Hardness index	0.004	0.640***	-0.395*	0.252	-0.028	0.185	-0.731***
Milling performances							
Flour yield	-0.030	0.507*	-0.042	-0.109	0.225	0.355	-0.517**
Proportion of break flour	-0.249	-0.380	0.389*	-0.495***	0.068	-0.240	0.390*
Physico-chemical Properties of flour							
Average particle size	0.091	0.636***	-0.487*	0.293	0.081	0.441*	-0.530**
Ash	0.051	0.480*	-0.640***	0.535**	-0.285	-0.125	-0.305
Damaged starch	0.036	0.706***	-0.404*	0.205	0.074	0.288	-0.747***
Water retention capacity	-0.287	0.322	-0.401*	0.236	-0.201	-0.221	-0.413*
Lightness of flour	0.116	-0.651***	0.528**	-0.197	-0.041	-0.253	0.619***
Protein	0.249	0.725***	-0.879***	0.687***	0.073	0.416*	-0.434*
Amylose	-0.093	-0.363	0.547**	-0.456*	-0.152	-0.362	0.212
SDS-sedimentation volume	0.076	0.469*	-0.126	0.136	0.324	0.210	-0.659***
Dough Rheology							
Absorption of mixograph	0.442*	0.571**	-0.727***	0.769***	0.181	0.608***	-0.449*
Mixing time of mixograph	0.493*	0.183	0.017	0.211	0.264	0.198	-0.351
Maximum height of dough	0.589**	0.115	0.072	0.167	0.212	0.286	-0.196

[a]* indicates significance at 0.05 level, ** at 0.01 level, and *** at 0.001 level.

Table 5 shows the relationships among grain characteristics, milling performances, physic-chemical properties and end-use qualities of Korean wheat cultivars with *Pina-D1a/Pinb-D1b* and *Pina-D1b/Pinb-D1a.* Loaf volume of bread was negatively correlated with water retention capacity and positively correlated with mixing time of mixograph and maximum height of dough. Properties of noodle dough sheet were significantly correlated with protein content and optimum water absorption of mixograph. Ash content and lightness of flour were also correlated with lightness of noodle dough sheet. Hardness of cooked noodles was correlated with flour milling performances, protein content and optimum water absorption of mixograph. Springiness of cooked noodles was positively correlated with flour yield, although springiness showed no significant relationships with grain characteristics, milling performances and physico-chemical properties of wheat flours with all different puroindoline genotypes. Cohesiveness of cooked noodles was correlated with water retention capacity and optimum water absorption of mixograph. Cookie diameter showed no significant relationships with grain characteristics, milling performances and physico-chemical properties of wheat flours.

Table 5. Correlation coefficients among grain characteristics, milling performances, physico-chemical properties and end-use qualities of Korean wheat cultivars with *Pina-D1b/Pinb-D1a* and *Pina-D1a/Pinb-D1b*

Parameters	*End-use quality*						
	Bread loaf volume	White salted noodles					Cookie diameter
		Dough sheet		Texture of cooked noodles			
		Thickness	Lightness	Hardness	Springiness	Cohesiveness	
Grain characteristics							
Test weight	0.056	0.349	-0.236	0.366	-0.127	-0.068	-0.149
1000-kernel weight	0.088	-0.209	0.003	-0.181	0.077	0.270	0.060
Hardness index	-0.292	0.223	-0.110	0.013	-0.152	-0.364	-0.433
Milling performances							
Flour yield	-0.094	0.024	0.448	-0.512*	0.496*	0.145	-0.008
Proportion of break flour	-0.177	-0.371	0.412	-0.573*	0.283	-0.119	0.362
Physico-chemical Properties of flour							
Average of particle size	-0.065	0.135	-0.028	-0.146	0.121	0.404	0.134
Ash	-0.033	0.326	-0.557*	0.440	-0.533*	-0.308	-0.132
Damaged starch	-0.052	0.423	-0.100	-0.140	0.210	0.051	-0.470
Water retention capacity	-0.542*[a]	-0.023	-0.246	0.081	-0.412	-0.581*	0.034
Lightness of flour	0.264	-0.368	0.517*	0.010	-0.199	-0.125	0.210
Protein	0.157	0.712**	-0.854***	0.592*	0.074	0.413	-0.191
Amylose	-0.131	-0.271	0.562*	-0.467	-0.161	-0.382	-0.172
SDS-sedimentation volume	0.081	0.012	0.500*	-0.164	0.414	-0.050	-0.293
Dough Rheology							
Absorption of mixograph	0.272	0.519*	-0.626**	0.762***	-0.008	0.568*	-0.014
Mixing time of mixograph	0.605**	-0.067	0.409	0.125	0.073	0.048	-0.149
Maximum height of dough	0.721***	-0.135	0.408	0.019	0.135	0.205	0.061

[a]* indicates significance at 0.05 level, ** at 0.01 level, and *** at 0.001 level.

3.4. Discussion

Most Korean wheat cultivars were shown to have *Pina-D1a*/*Pinb-D1b*, which was similar to the genetic variations of CIMMYT lines, North American, Europe, China and Australia (Lillemo and Morris 2000, Morris *et al.* 2001, Cane *et al.* 2004, Xia *et al.* 2005, Chen *et al.* 2006, Lillemo *et al.* 2006, Pickering and Bhave 2007). There were no significant differences in grain characteristics and milling performances of Korean wheats with *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b* genotypes, although these genotypes showed higher 1000 kernel weight, hardness index and flour yield than Korean wheats with *Pina-D1a/Pinb-D1a* genotypes. Nagamine *et al.* (2003) reported that *Pina-D1a/Pinb-Db* had higher flour yield than *Pina-D1a/Pinb-Da* in 110 doubled haploid lines, although there was no significant difference in 1000 kernel weight. Hard wheats with *Pina-D1a*/*Pinb-D1b* had significantly higher flour yield, break flour yield and milling score than wheats with *Pina-D1b*/*Pinb-D1a* in recombinant inbred lines of hard red spring wheats (Martin *et al*., 2001) and Australian wheats (Eagles *et al*., 2006). However, there was no significant difference in flour yield and proportion of break flour between *Pina-D1a*/*Pinb-D1b* and *Pina-D1b*/*Pinb-D1a* genotypes in Korean wheats.

Hardness index of SKCS is widely accepted as the standard method to determine the single wheat kernel texture and is the most discriminating measure of the material properties of the wheat endosperm manifested by the action of the *Ha* locus (Moris *et al.* 1999, Osborne and Anderssen, 2003). Hardness index was significantly correlated with flour yield and the proportion of break flour in Korean wheats with all puroindoline genotypes, but no significant relationships were found in Korean hard wheats. However, hardness index was negatively correlated with flour yield in hard wheats (Ohm *et al*. 1998, Martin *et al.* 2001) and Chinese winter wheats (Zhang *et al*. 2005) and also negatively correlated with break flour yield in U.S. wheats with various puroindoline genotypes (Bettge and Morris 2000).

Korean wheats with *Pina-D1a/Pinb-D1a* genotypes showed different physico-chemical properties including particle size of wheat flours, flour color, ash, protein and damaged starch content, water retention capacity, SDS-sedimentation volume and mixograph properties from Korean wheats with *Pina-D1b*/*Pinb-D1a* and *Pina-D1a*/*Pinb-D1b* genotypes. Soft wheats showed lower average particle size of flour, ash and damaged starch content, and higher lightness of flour than hard wheats in doubled haploid lines from Japan and U.S. wheats with different puroindoline

genotypes (Bettege and Morris 2000, Nagamine *et al*. 2003). Korean wheats with *Pina-D1b*/*Pinb-D1a* had greater starch damage in milling processing than *Pina-D1a*/*Pinb-D1b* genotypes and the damage could have an effect on water absorption of rheological properties, which are in agreement with previous results (Cane *et al*. 2004, Eagles *et al.* 2006, Chen *et al.* 2007). There were no significant differences in any other physico-chemical properties and mixing properties between those Korean wheat cultivars. In hard red spring wheats and cultivated wheats in China, *Pina-D1a/Pinb-D1b* had lower ash content and brighter flour color than *Pina-D1b/Pinb-D1a* but there was no difference in mixing time of mixograph (Martin *et al*. 2001, Chen *et al.* 2007). In near-isogenic lines of puroindolines, no difference was found in ash content and water retention capacity between *Pina-D1a/Pinb-D1b* and *Pina-D1b/Pinb-D1a,* however, the former had lower damaged starch content and brighter color than the latter (Ma *et al*. 2009).

In Korean wheats, physical properties of flour including average particle size of flour, ash and damaged starch content, water retention capacity and lightness of flour showed significant relationships with hardness index or flour yield. There were also significant relationships between these physical properties in Korean wheats. Those results are in good agreement with previous results (Bettege and Morris 2000, Morgan *et al*. 2000, Martin *et al*. 2001, Nagamine *et al*. 2003, Zhang *et al*. 2005, Chen *et al*. 2007). Therefore, grain characteristics, especially hardness index of grain, could have influence on the milling performance and physical properties of Korean wheat flours.

Pina-D1b/Pinb-D1a genotypes had better bread quality in bread and Chinese steam bread than *Pina-D1a/Pinb-D1b* (Martin *et al.* 2001, Chen *et al.* 2007). However, there were no significant differences in bread volume among Korean wheats, although bread volume showed significant relationships with dough rheological properties. It should be considered to increase the frequency of *Pina-D1b/Pinb-D1a* genotypes in Korean wheat cultivars with strong gluten strength to improve bread-baking quality. In noodle making properties and texture of cooked noodles from Korean wheats, no significant differences were found among purondoline genotypes except for thickness of noodle dough sheet. Ma *et al.* (2009) reported that there were no significant differences in qualities for Chinese raw white noodles between *Pina-D1b/Pinb-D1a* and *Pina-D1a/Pinb-D1b.* They also proposed that *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* had slightly superior noodle qualities in comparison with other genotypes. However, Chen *et al.* (2007) reported that *Pina-D1b/Pinb-D1a* also had higher noodle score for dry white Chinese noodles than *Pina-D1a/Pinb-*

D1b and *Pina-D1a/Pinb-D1a*. Therefore, it should be considered to introduce various puroindoline genotypes such as *Pina-D1a/Pinb-D1e* and *Pina-D1a/Pinb-D1g* to improve noodle quality. Cookie diameter was influenced by hardness index, milling performances and physico-chemical properties, and especially by the particle size of flour, damaged starch content and SDS-sedimentation volume. *Pina-D1a/Pinb-D1a* had larger cookie diameter than other genotypes, which is in agreed with the report of Bettge and Morris (2000).

In Korean wheat cultivars, grain hardness, flour yield and physical properties of flour were mainly associated with the genetic variations of puroindolines. Hard wheat type, carrying *Pina-D1b* or *Pinb-D1b* alleles, showed higher grain weight, flour yield, average of particle size of flour, damaged starch content and water absorption than soft type wheats contained with *Pina-D1a* and *Pinb-D1a* alleles. However, end-use qualities have not been affected by the allelic variations in puroindolines albeit significant differences were found in dough rheological properties. Korean wheats should be introduced various puroindoline genotypes, like *Pinb-D1e* and *Pinb-D1g* alleles and increased the frequency of *Pina-D1b* allele to improve flour yield and qualities of bread and noodles and to overcome the narrow genetic background in puroindolines.

Chapter-4

General discussion

Protein modulation and the quantities of expressed proteins can define the various functional stages of the cell. Recently, protein identification is an almost routine requirement. However, reliable techniques for quantifying unmodified proteins (including those that escape detection under standard conditions, such as protein isoforms and membrane proteins) as well as the detection and quantification of posttranslational protein modifications are still far from being a matter of routine. Hence, there is a need for a profound understanding of the principles underlying modern protein analysis, in order to apply and improve established and novel methods successfully. This gives a detailed survey of development of protein analysis method and its application to the improvement of wheat seed quality addressing a simple and relatively rapid sample preparation method for Cleveland peptide mapping.

The application of Cleveland peptide mapping helps to identify the seed embryo proteins separated by 2-dimemsional electrophoresis (2-DE) in rice proteome analysis. Because of the accuracy between the experimental and in silico masses is critical to obtaining the correct protein identification, it is best to acquire the peptide map on a high mass measurement accuracy instrument, such as a time-of-flight (TOF) configuration. The greater the number of matches betweene experimental and database peptide masses, the higher the confidence in the protein's identification. Although, peptide-mass fingerprinting is a high-throughput method, I could not easily identify all the rice proteins or genes by this method. Because the complete database information on rice, is not yet available and many proteins are post-translationally modified. Therefore, at present, the improved peptide mapping method as I postulated here is considered to be very useful in rice and wheat proteome analysis that was successfully analyzed, especially for blocked proteins.

Plant proteomics is a relatively new research field focused on the large-scale functional analysis of proteins extracted from intact plants, particular plant organs, tissues, individual cells, subcellular organelles and/or separated sub-organellar structures. There has been a rapid progress in plant proteomics with plants in cluding wheat, with specific aspects such as establishing 2-DE reference maps of expressed proteins in developing wheat lemma and leaf.

Among the three major food crops (rice, wheat and maize), wheat is unique in accumulating gluten proteins in its grains. Of these proteins, the high and low molecular weight glutenin subunits (HMW-GSs and LMW-GSs) form glutenin macropolymers that are vital for the diverse end-uses of wheat grains. In this book, we emphasizes to develop a rapid MALDI-TOF-MS method to

determine accurate molecular weights of single HMW glutenin subunits from mixture samples. Wheat is one of the most important cereal crops worldwide, in terms of production and utilization. It is a major source of energy, protein, and dietary fiber in human nutrition. Wheat is consumed in many different forms, mainly as chapati, bread, noodles, macaroni, spaghetti, cakes, pizzas, and doughnuts. The ability of wheat flour to be processed into different foods is largely determined by the gluten proteins. The gluten proteins constitute up to 80% to 85% of total flour protein, and confer properties of elasticity and extensibility that are essential for functionality of wheat flours. Consequently, the allelic variation in gluten have been widely studied over a period, in order to determine their structures and properties and to provide a basis for manipulating and improving end use quality, and to provide useful information for improving wheat quality in Korean wheat breeding programs.

Grain texture is a major characteristic and a determinant of end product quality, especially important in baking and noodle making. Grain hardness is caused by the presence of puroindoline genes (*Pina* and *Pinb*). This chapter has focused on the relationships among puroindoline genotypes, grain properties and milling performance, as well as determined the influence of puroindoline genotypes on physico-chemical properties of flour and qualities of bread, white salted noodles, and sugar-snap cookies made from Korean wheat cultivars with a view to provide useful information for improving wheat quality in Korean wheat breeding programs. A simple, efficient and rapid method were developed to obtain protein sequence information for the blocked proteins. Peptide mass fingerprinting determined by matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDITOF-MS) was used to identify the rice proteins separated by two-dimensional electrophoresis (2-DE). Although, peptide-mass fingerprinting is a high-throughput method, still the method has drawbacks. Because the complete database information on rice, is not yet available and many proteins are post-translationally modified. Therefore, at present, the improved peptide mapping method as we postulated here is considered to be very useful in rice proteome analysis, especially for blocked proteins. A state-of-the-art proteomic approach was employed to identify lemma proteins and the diversity of glutenin genes from wheat cultivars. The diverse glutenin subunits in wheat cultivars were identified and compared the gluten isoforms among different wheat cultivars according to quality. However, the findings provide an insight on the quality improvement in wheat crop. In addition, dough rheological properties as well as end-use quality were addressed to determine the effects of *Glu-1* and *Glu-3* alleles on those

characteristics in Korean wheat cultivars using gel-based approaches.

Conducted PCR analysis instead of protein analysis, to validate proteomic data in wheat genotypes due to challenges in separating storage proteins. Difficulty in matching peptides to gene sequences emphasizes the need for comprehensive information on flour protein genes, attainable through wheat genome sequencing initiatives. This study highlights the importance of advanced proteomic techniques in understanding crop proteomes for enhancing wheat and product quality. The study utilizes a rapid sample preparation method for Cleveland peptide mapping, emphasizing high mass accuracy with orbitrap mass spectrometry. Knowledge sharing in proteomic analysis supports the commitment to improve Korean wheat breeding program quality. Aspiring to develop advanced protein analysis methods demonstrates dedication to education and innovation for wheat seed quality improvement in Korea. Furthermore, the research provides insights into genetic and protein factors influencing wheat properties, guiding future research and practices for enhancing wheat seed quality and productivity**.** Since I returned from Japan, I conducted research on proteome. It was applied for the first time in Korea's agricultural sector. Numerous students and researchers, both domestic and international, as well as employees from private companies, have received instruction in proteomics technology from my laboratory (Plant Breeding and Functional Proteomics, Chungbuk National University) under my guidance. Currently, all of them are applying this protein analysis technique to enhance crop quality and productivity, thereby making contributions to the field of Agriculture in Korea, including crop, breeding, quality research, and plant physiology. Based on proteomics I published numerous articles and book chapters in international journals. In addition, there are many contributions in the field of Agriculture for improving the quality and cultivar development of wheat in Korea by using the proteome analysis that I learned from Japan. Now protein analysis methods are being employed in various research organization in Korea. For example, RDA, University, Private companies.

(i) Protein analysis technology was used to develop varieties resistant to pre-harvest sprouting (PHS) (Abu *et al.* 2009), aiming to reduce the impact of *Fusarium* head blight (FHB) on both quantity and quality (Shin *et al*. 2011).

(ii) Puroindoline genotypes have been studied in selection for wheat varities with optimal processability, including low amylose content for noodles, increased gluten for bread, reduced polyphenol levels for color improvement, and enhanced milling properties (s). The cultivation of these improved wheat varieties has led to an annual increase in the

production of high-quality wheat in Korea (Park *et al.* 2009).

(iii) A novel y-type subunit was discovered at the *Glu-D1* locus, originating from a Korean wheat line that carried the *Glu-D1y* allele obtained from F9 lines crossed by Keumkang, a leading Korean cultivar with the *Glu-D1d* allele, and Chinese Spring with the *Glu-D1a* allele. This specific line was named JB20, and the newly identified y-type subunit was designated Glu-1Dy12.K. The profiling of this subunit was determined using SDS-PAGE combined with 2-DE (2-dimensional electrophoresis). Additionally, LTQ-FT-MS (linear ion-trap and Fourier-transform mass spectrometry) and DNA sequencing were employed to understand both the protein function of Glu-1Dy12.K and the nucleotide sequences associated with it. The application of both proteomic and genomic analysis, practiced in various research organizations in Korea, facilitated the successful output of this technology (Cho *et al.* 2017).

(iv) I, with colleagues, presented findings on a Korean wheat landrace containing the Bx7OE subunit (IT166460) and conducted a molecular characterization of the Bx7OE subunit using SDS–PAGE combined with 2-DE, RP-HPLC, and DNA sequencing. Notably, the majority of Korean wheats exhibit limited genetic variation in glutenin composition that are associated with inferior bread quality. Therefore, IT166460 could serve as a valuable resource to enhance the bread-making quality within the Korean wheat breeding program, a possibility identified through proteomic analysis (Cho *et al.* 2017).

(v) Physicochemical properties of Korean wheat flours were evaluated through proteomic analysis to determine the effect of flour characteristics on yellow alkaline noodles by comparing commercial and imported wheat flours. Optimum water absorption, thickness, and color of noodle dough significantly correlated with protein content-related parameters of flour. There are many researchers are engaging with proteomic research to produce good quality wheat in Korea (Heo *et al.* 2012).

(vi) Influence of Allelic Variations of Glutenin and Puroindoline on Flour Composition, Dough Rheology and Quality of White Salted Noodles from Korean Wheat Cultivars: Most Korean wheat cultivars and advanced lines have 2.2+12 subunits in *Glu-D1* locus and they showed inferior noodle color to commercial noodle wheat flours and lower loaf volume and harder crumb firmness than commercial bread flours in spite of the similar protein content. However, there was little information about the association of allelic

variations in glutenin including HMW-GS and LMW-GS with end-use properties of Korean wheat cultivars. Therefore, a study was conducted to elucidate the relationship between dough rheological properties and end-use characteristics of Korean wheat cultivars through the comparison with commercial flours and imported wheat flours, to determine the effects of *Glu-1* and *Glu-3* alleles on those characteristics of Korean wheat cultivars, and to provide useful information for improving wheat quality in Korean wheat breeding programs (Shin *et al.* 2012).

In conclusion, this study focuses to the importance of advanced proteomic techniques in gaining a deeper understanding of crop proteomes and their implications for crop improvement and product quality enhancement. The researches presented here provide valuable insights into the genetic and protein-level factors that influence the properties of wheat, offering promising avenues for future research and agricultural practices aimed at improving wheat quality and productivity.

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