

N-Terminal Amino Acid Sequence Analysis of Endosperm Proteins in Japanese Hexaploid Wheat

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ABSTRACT

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Semidry electroblotting is convenient and allows a rapid and efficient protein transfer from two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) gels onto sequencer stable supports for protein microsequence analysis in a gas-phase sequencer. Using this technique, I determined the amino acid sequences of the endosperm proteins in Japanese hexaploid commercial wheats (*Triticum aestivum*). Based on sequence determination of the Japanese hexaploid wheats, the endosperm protein could be easily characterized. Wheat endosperm protein, extracted in the presence of 2-mercaptoethanol and SDS, fractionated into many protein polypeptides using 2D-PAGE under dissociating conditions. These components

were grouped into HMW glutenin subunits, α -, β - or γ -gliadins, and novel protein polypeptides by using the N-terminal amino acid sequences. The novel endosperm protein polypeptides were detected, and two new types of N-terminal amino acid sequences have been found for protein polypeptides. These polypeptides have much faster electrophoresis mobility during 2D-PAGE and are therefore probably a much smaller size than any other peptides of endosperm protein groups found in hexaploid wheat. Ten protein polypeptides have been purified from cultivars of Japanese wheat. Some differences in the contents of amino acids for four protein polypeptide spots were apparent in Japanese wheat.

The predominant proteins of the wheat endosperm proteins are described here as prolamins (Shewry et al 1984) because of their high content of proline and glutamine (Kasarda et al 1976). In the developing endosperm, they are deposited in membrane-bound protein bodies (Field et al 1983). These proteins are subdivided into glutenin and gliadin. Glutenin is radically different from gliadin and occurs as large disulfide-linked molecules or aggregates. It elutes with the void volume upon gel-filtration chromatography (Payne et al 1979). The gliadin fraction is a complex mixture of simple polypeptides. They do not participate in intermolecular disulfide bond formation, and they are not excluded from gel filtration media designed to sieve molecules at MW < 80,000 (Nakamura et al 1990).

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) has been extensively used for separation and characterization of proteins (Lafandra et al 1985; Payne et al 1985). Commonly, the first-dimension separation occurs in a gel cylinder or strip, which is then positioned across the top of a second slab gel for separation in the second dimension. In this way, almost any two one-dimensional electrophoretic techniques can be combined to improve resolution of a protein mixture. Amino-terminal sequences of endosperm proteins were examined to reveal evolutionary relations, aid in classification, and relate their structures and properties (Bietz et al 1977).

Prolamins are the major storage proteins in most cereal seeds and as such are an important source of dietary protein. In addition, the prolamins of wheat are the major components of gluten, the properties of which determine the quality of wheat flour for various technological processes including breadmaking quality (Shewry and Tatham 1990). The starchy endosperm of mature wheat grains contains several types of protein including the storage proteins glutenin, gliadin, globulin, and albumin proteins and enzymes that survived from the metabolically active endosperm of the developing grain, and structural proteins, such as those in membranes.

A novel method for protein microsequencing has been rapidly developed during the last 15 years. Several authors have reported that the wheat endosperm protein was extracted from wheat flour and amino acid sequences were extracted (Thompson et al 1983; Forde et al 1985; Halford et al 1987, 1992; Anderson et al 1989; Hirano 1989; Kasarda 1989; Hirano and Watanabe 1990; Reddy and Apples

1993; Mackie et al 1996; Bleukx et al 1998; Masci et al 1999; Ovidio et al 1999). In this method, subnanomole amounts of protein are first separated by one-dimensional (1D) or 2D-PAGE, electroblotted from the gel onto sequencer-stable membrane supports, and finally sequenced directly with a gas-phase sequencer. The sample preparation procedure, consisting of only two steps, high resolution of 2D-PAGE, and electroblotting, effectively prevents protein losses and modifications inherent to multistep sample preparation and often permits the purification of proteins that could not easily be purified by conventional column chromatography, including HPLC.

In this article, the 2D-PAGE protein obtained by isoelectric focusing (IEF) in the first dimension \times SDS-PAGE in the second 2D-PAGE procedures has been subdivided further according to the biochemical and genetic properties of the proteins. To overcome this limitation, a detailed analysis of endosperm proteins from a single cultivar is required. At present, no systematic effort has been reported on the characterization of a gliadin family from a single cultivar in Japan. The only extensive analysis in this direction is the one done on cvs. Norin 1, Norin 2, Norin 3, Norin 4, and Chinese-Spring, where a total 10 protein peptide spots have been sequenced.

To extend knowledge of endosperm proteins and particularly of genes related to protein characteristics of Japanese hexaploid wheat, I have started a research project to characterize the complete gene set of a Japanese wheat cultivars by following two strategies. The allelic variation in the HMW glutenin subunit loci of *Glu-1* in the Japanese hexaploid wheat cultivars is unique throughout the world although, for these cultivars, there are only 17 glutenin subunit compositions (Nakamura et al 1999). The N-terminal amino acid sequences of 10 individual protein spots isolated from wheat cultivars are reported here. These results give further information on the genetic and biochemical relationships within this important group of wheat endosperm proteins.

MATERIALS AND METHODS

In this study, electroblotted proteins were separated by 2D-PAGE and their N-terminal amino acid sequences determined. Japanese hexaploid wheat cvs. Norin 1, Norin 2, Norin 3, Norin 4, and Chinese-Spring used in this study were taken from the collection maintained at the National Agriculture Research Center, Tsukuba, Japan. Electroblotting of proteins for microsequencing analysis employed a semidry blotting apparatus, developed by Kyhse-Andersen. Proteins electroblotted onto polyvinylidene difluoride (PVDF) can easily be detected with Coomassie Brilliant Blue, Amino Black, or Ponceau S. I preferred the PVDF (pore size 0.45 μ m) due to its

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characteristics in the blotting for microsequencing. Most of the proteins were well bound onto PVDF and could easily be detected by Coomassie Blue staining in this study. To complete polymerization, a polyacrylamide gel was usually prepared 1 or 2 hr before use. However, the gel could be used a few hours after initiation of polymerization without any particular problems in the separation and sequencing of proteins. Protein samples for 2D-PAGE were prepared according to the standard methods. Alternatively, pyridylethylation can be done in gas phase on the immobilizing matrix after electroblotting. The thickness and concentration of polyacrylamide gels affected electroblotting efficiency. In most cases, a 2-mm-thick gel was most suitable.

Proteins separated by 2D-PAGE were electroblotted in a semidry blotting apparatus and sequenced by a gas-phase sequencer. Total endosperm proteins were isolated from wheat flour in Japanese Norin 1 by a gas-phase sequencer. Three buffer solutions were used in electroblotting: solution A consisted of 0.3M Tris, 20% (v/v) methanol; solution B was 25 mM Tris, 20% (v/v) methanol; and solution C was 25 mM Tris, 40 mM 6-amino-*n*-caproic acid, 20% (v/v) methanol. Blotting filters and filter paper (Whatman 3MM) were trimmed to the dimensions of the gel. PVDF or siliconized glass fiber filter (SGF) was soaked in 100% methanol for 10 sec and subsequently in solution C for 5 min with shaking. Polybrene-coated glass fiber (PGF) was washed carefully with 200 mL of redistilled water to remove excess polybrene and soaked in solution C for 5 min with shaking. After completion of 2-D PAGE, the gel was soaked in 100 mL of solution C for 10 min with gentle shaking to reduce the amount of glycinate contained in the gel. A total of ~200 mL each of A, B, and C was poured into three separate stainless trays. Two pieces of filter paper were soaked in each tray and shaken gently for 10 min to wash out the contaminants contained in the filter papers. Any excess solution in the filter papers and gel was removed. The 2D-PAGE involved two separate first dimensions and a common second dimension. Most proteins could be fractionated in the procedure using IEF in the first dimension and SDS-PAGE in the second. The method, originally described

by O'Farrell (1975) and O'Farrell et al (1977), has been modified further for the separation of wheat endosperm proteins (Nakamura et al 1990). Samples for extraction weighed 30 mg and consisted of several grains with their embryos discarded. Acrylamide, N,N'-methylenebisacrylamide, SDS, and urea were purchased from Bio-Rad (Richmond, CA). Ampholine carrier ampholytes were from LKB (Bromma, Sweden). Acetonitrile and methanol were HPLC analytical reagent grade. Reagents and solvents for amino acid sequence analysis were purchased from Wako Pure Chemicals (Osaka, Japan) and Applied Biosystems Japan (Tokyo). Glass-fiber (GF) filter and PVDF (Immobilon, pore size 0.45 μm) were purchased from Whatman (Maidstone, Kent, UK) and Millipore (Bedford, MA), respectively. Filter paper (3MM) was obtained from Whatman. Bovine α-lactalbumin and β-lactoglobulin were from Pharmacia (Uppsala, Sweden) and Applied Biosystems (Foster City, CA), respectively. The pure spots in wheat endosperm proteins separated by 2-D PAGE were electroblotted in a semidry blotting apparatus and sequenced by a gas-phase automatic sequencer with a Applied Biosystems model 477A.

RESULTS

N-terminal amino acid sequencing (Kasarda et al 1984; Shewry et al 1984) and DNA sequencing of gliadin gene sequences cloned in bacteria revealed four distinctive groups: α-, β-, γ- and ω-gliadins (Payne et al 1985). Upon reduction, the aggregates dissociate into several dozen protein spots that group into HMW and novel protein polypeptide spots by two-dimensional electrophoresis. By the 2D-PAGE, the novel protein polypeptides subdivided. In addition to the major storage proteins, there are myriad additional, minor proteins, which together make a significant contribution to total endosperm protein.

Amino acid compositions of 10 protein polypeptides were examined to reveal the gliadin polypeptides with lower molecular weight than HMW glutenin subunits. Numerous protein components are present, many LMW and not seen clearly in total protein extracts (Fig. 1).

TABLE I
N-Terminal Amino Acid Sequences of Purified Wheat (*Triticum aestivum*) Endosperm Proteins in Japanese Wheat Cultivar Norin 1

Protein Spot	Identified Residues	N-Terminal Amino Acid Sequences ^a	Protein Polypeptide
1	18	<u>T G P Y</u> (T) <u>Y A G M G L P I N X L X G</u>	Novel protein N ₁
2	20	<u>T G P Y X Y P G M G L P S N P X E G X Q</u>	Novel protein N ₂
3	6	<u>R T A X E P</u>	Novel protein N ₃
4	8	<u>R I A</u> (S) <u>E</u> (Q) Q (H)	Novel protein N ₄
5	10	<u>V R V P V P Q L Q P</u>	α-, β-gliadin
6	17	<u>V R V P V P Q L Q P Q N P S Q</u> (Q) Q	α-, β-gliadin
7	20	<u>N M Q V D P S G Q V Q W P Q Q X X X P Q</u>	γ-gliadin
8	8	<u>V R V P V P Q L</u>	α-, β-gliadin
9	11	<u>E G E A S X Q L Q X Q</u>	HMW ^b glutenin subunit 2
10	9	<u>E G E A S G E L Q</u>	HMW glutenin subunit 12

^a Sequences aligned to maximize homology, resulting in gaps in those for seed storage protein components. Underlines indicate regions of sequence identity. Notation of residue numbers follows standard single letter abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; G, glycine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; Y, tyrosine; V, valine; W, tryptophan; Z, glutamine; X, unidentified.

^b High molecular weight.

TABLE II
Comparison of Fraction of the Endosperm Protein Spots in Wheat (*Triticum aestivum*) by 2D-PAGE (IEF × SDS-PAGE)^a

Cultivar	Protein Spot Number ^b									
	Novel Protein Polypeptide				Gliadin				HMW Glutenin Subunit ^c	
	1	2	3	4	5	6	7	8	9	10
Norin 1	+	+	+	+	+	+	+	+	+	+
Norin 2	+	+	+	+	+	+	+	+	+	+
Norin 3	-	-	-	+	+	+	+	+	+	+
Norin 4	+	-	-	-	+	+	-	-	+	+
Chinese-Spring	-	-	-	-	-	+	-	-	+	+

^a 2D-PAGE = two-dimensional polyacrylamide gel electrophoresis; IEF = isoelectric focusing.

^b Present = + and absent = -.

^c High molecular weight.

The 2D-PAGE fractionation of the total endosperm proteins of Norin 1 is shown in Table I. The 2D-PAGE proteins were divided into areas according to the biochemical and genetic properties of the proteins, and results were similar, although more detailed, than those published previously (Payne et al 1985). The subdivision of the map into HMW glutenin subunit, LMW glutenin subunit, gliadin, albumin, and globulin is based on Jackson et al (1983). During a survey of hexaploid wheat cultivars, several Japanese modern cultivars (Norin 1, Norin 2, Norin 3, and Norin 4) possessed the novel four protein polypeptides of much faster mobility in 2D-PAGE (Table II). These protein polypeptides were not reported previously.

The four novel protein polypeptides were not fully characterized biochemically and can not yet be included in the classification scheme shown in Fig. 1 and Table I. They are present in protein bodies in the developing endosperm, but they are endosperm proteins that do not fall clearly into the glutenin, gliadin, albumin, or globulin groups.

The sequence results for four novel protein polypeptides (Table I or Fig. 1). The novel protein polypeptide named N_1 in this study has the sequence $\text{NH}_2\text{-Thr-Gly-Pro-Tyr-(Thr)-Tyr-Ala-Gly-Met-Gly-Leu-Pro-Ile-Asn-Leu-Gly-}$. The novel protein polypeptide named N_2 in this study also has the same sequence for 12 residues, $\text{NH}_2\text{-Thr-Gly-Pro-Tyr-, -Tyr-, -Gly-Met-Gly-Leu-Pro-, -Asn-, -Gly-}$, except that two residues could not be identified. The protein spots 1 and 2 have regions of homology with $\text{NH}_2\text{-T (threonine) - G (glycine) - P (proline) - Y (tyrosine)-, - Y (tyrosine) -, - G (glycine) - M (methionine) - G (glycine) - L (leucine) - P (proline)-, -N (asparagin)-, -G (glycine)-}$, in agreement with 12 residues, respectively. Therefore, I have determined this two-protein polypeptide. The determined sequence of the novel protein polypeptide named N_3 in this study was $\text{NH}_2\text{-Arg-Thr-Ala-Glu-Pro-}$; the novel protein polypeptide named N_4 in this study also has $\text{NH}_2\text{-Arg-Ile-Ala-(Ser)-Glu-(Gln)-Gln-(His)-}$. Thus, four novel protein polypeptides (N_1 , N_2 , N_3 , and N_4) in this study differ significantly from endosperm proteins such as glutenin, gliadin, albumin, or globulin groups sequences (Bietz et al 1977; Kasarda et al 1983, 1984; Shewry et al 1984, 1989, 1990; Halberd 1985; Rafalski 1986; Anderson and Green 1989; Anderson et al 1989; Masci et al 1999).

The purified protein polypeptides were identified by their mobility on the gel by 2D-PAGE. Ten fractions gave major or minor protein peptides on 2D-PAGE (Fig. 1). They are monomeric proteins. Amino acid compositions of 10 protein polypeptide spots preparations are presented in Table I. It contains at least four types of proteins, usually referred to as α -, β -, γ - and ω -gliadins. Although the novel protein N_1 , N_2 polypeptide preparations are rich in tyrosine (Y), proline (P), and glycine (G), and the novel protein N_3 , N_4 polypeptide preparations are also rich in arginine (R), alanine (A), and glutamic acid (E), some differences in the proportions of those amino acids are observed from other endosperm protein polypeptides (glutenin, gliadin, albumin, and globulin). The α -, β -gliadin polypeptides preparations are rich in glutamine (Q), proline (P), and valine (V). The γ -gliadin polypeptides preparations also are rich in glutamine (Q), proline (P), and valine (V). The HMW glutenin polypeptides preparations are rich in glutamine (Q), glutamic acid (E), and glycine (G). The novel protein polypeptide N_1 , N_2 has regions of homology ($\text{NH}_2\text{-threonine(T)-glycine(G)-proline(P)-and tyrosine(Y)-}$), but these sequences do not correspond to the predominant N-terminal amino acid sequence found in the sequence of the α -, β -gliadin groups (Kasarda et al 1984). Japanese cvs. Norin 1 and Norin 2 possessed the novel protein polypeptides N_1 , N_2 , N_3 , and N_4 . On the other hand, Chinese-Spring does not possess these four novel protein polypeptides (Table II). Norin 3 has only the novel protein polypeptide N_4 and Norin 4 has only the novel protein polypeptide N_1 . Norin 1 and Norin 2 possessed all 10 protein spot numbers ,1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. Norin 1, Norin 2, Norin 3, Norin 4, and Chinese-Spring have the same HMW glutenin subunit composition, protein spot number 9 and 10.

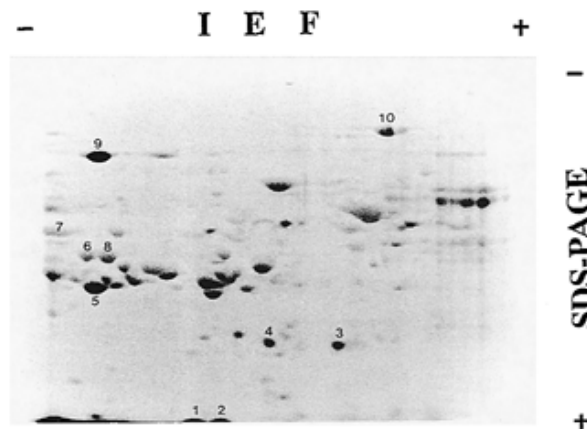


Fig. 1. Fractionation of endosperm proteins by isoelectric focusing (IEF) \times sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of Japanese wheat cultivar Norin 1.

DISCUSSION

At first, the aim of this study was to reveal the gliadin polypeptides in Japanese modern cultivars, but I determined novel endosperm protein polypeptides with very low molecular weight (slot 1 and 2) on the same gel in these analyses.

Japanese hexaploid wheat cultivars are unique in terms of their HMW glutenin subunit composition because two rare alleles are present in Japanese wheat (HMW glutenin subunits 13+19 encoded at the *Glu-B1* allele and subunits 2.2+12 encoded at the *Glu-D1* allele) (Nakamura 1999). New types of N-terminal amino acid sequence were found in Norin 1, Norin 2, Norin 3, and Norin 4. Altogether, five types of N-terminal amino acid sequence were found: two new types and three other sequences corresponding to HMW-glutenin types, α -, β -gliadin type, and γ -gliadin type, as previously reported (Bietz et al 1977; Kasarda et al 1983, 1984; Shewry et al 1984, 1989, 1990; Halberd et al 1985; Rafalski 1986; Anderson and Green 1989; Anderson et al 1989; Masci et al 1999). These four cultivars (Norin 1, Norin 2, Norin 3, and Norin 4) have four HMW glutenin subunits (7+8, 2+12); none of them unique. However, these Japanese cultivars have the novel protein polypeptides of endosperm protein with very fast mobility in 2D-PAGE. I have named the two novel protein polypeptides N. Amino acid sequencing has great potential for analysis of the evolutionary relationships among endosperm protein components and genes coding them, but this technique is only beginning to be applied in the study of those proteins in Japan. So far, no cereal grain storage protein has been sequenced completely, even though the complete sequence of more than a thousand proteins has been determined. Automated Edman degradation reveals a high degree of homology among HMW glutenin polypeptides, α -, β -gliadin polypeptides, and γ -gliadin polypeptides.

These novel protein polypeptides are not major endosperm proteins such as glutenin, gliadin, albumin, and globulin. The differences between these novel protein polypeptides and other protein polypeptides must be few in number and must occur well past the amino-terminal region. This explains how a mixture of novel protein polypeptides may be identical for the first 20 residues at best. Comparison of my data to the N-terminal amino acid sequence of novel protein polypeptides suggest that there are relatively few differences between endosperm proteins from Japanese commercial wheat (*T. aestivum*) cultivars. It is possible that this sequence is specific for Japanese wheat, but this possibility needs further investigation. The relationship between four protein polypeptides and wheat quality aspects has not yet been determined. The variation in HMW glutenin subunit composition in Japanese hexaploid wheat is very different from that of the cultivars throughout the world (Nakamura 1999). Therefore, the endosperm protein components of Japanese wheat is unique and is of great interest to Japanese

breeders or cereal chemists. The possible effects on bread- or noodle-making quality of the named novel protein polypeptides have not yet been determined and their function remains unclear. Further sequencing will be necessary to determine the extent to which repeated sequences occur in novel protein polypeptides. I was unable to extend our N-terminal sequences beyond ≈20 residues at best. I think that this results from limitations inherent in the nature of those novel protein polypeptides. This process probably occurred in a stepwise manner with larger, possibly diverged, sequences being duplicated in later steps, and eventually involved duplication of complete genes. In this study, by employing the electroblotting and microsequencing technique, the data file consisting of the peptide map on the gel and the N-terminal sequences of the mapped proteins, called a protein library (Hirano 1989), could be constructed easily from only wheat flour. The protein library in particular will be useful for the synthesis of an oligonucleotide probe, which will be used for the cloning of the gene encoding the protein mapped on the gel and for the identification of the coding region and reading frame of the gene. The protein library will be used to define post-translational modifications of the protein synthesized by the gene in the future.

The completely novel opportunity for researching the relationships of interaction and composition of the proteins on wheat quality is being revealed by the construction of modified genes for gluten polypeptides, such as those reported by Shani et al (1992). Expression of such novel genes in a heterologous system offers the opportunity to test the functional properties in wheat quality for subunits processing very different structures. For example, glutenin polypeptides are becoming available with a longer or shorter repetitive region in the middle of the polypeptide. These approaches are expected to contribute significantly toward formulating models of the ideal structure for the range of gluten proteins. Recent advances in the transformation of wheat (Vasil et al 1991; Anderson et al 1994; Brettell et al 1994) will enable the direct testing of such models by the incorporation of the modified wheat genes and the subsequent commercial availability of hexaploid wheat with improved wheat quality.

The novel protein polypeptides described here add to the known electrophoretic variation in endosperm proteins among Japanese hexaploid wheat cultivars and will be useful in cultivar identification and in determining the homogeneity of genotypes within modern cultivars and lines. The effect of four novel protein polypeptides and four gliadin polypeptides on gluten will also be investigated, and it has been possible to rank them in terms of their contribution to this character. The results reported in this study indicate that the N-terminal amino acid sequence is a powerful tool to characterize the unique protein components in Japanese hexaploid wheat.

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LITERATURE CITED

Anderson, O. D., and Greene, F. C. 1989. The characterization and comparative analysis of HMW glutenin genes from genomes A and B of hexaploid wheat. *Theor. Appl. Genet.* 77:6817-6832.

Anderson, O. D., Greene, F. C., Yip, R. E., Halford, N. G., Shewry, P. R., and Malpica-Romero, J. M. 1989. Nucleotide sequence of the two high-molecular-weight glutenin genes from the D-genome of a hexaploid bread wheat, *Triticum aestivum* L. cv. Cheyenne. *Nucleic Acids Res.* 17:461-462.

Anderson, O. D., Blechl, A. E., Greene, F. C., and Weeks, J. T. 1994. Progress towards genetic engineering of wheat with improved quality. Pages 3-9 in: *Improvement of Cereal Quality by Genetic Engineering*. Plenum Press: New York.

Bietz, J. A., Huebner, F. R., Sanderson, J. E., and Wall, J. S. 1977. Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. *Cereal Chem.* 54:1070-1083.

Bleukx, W., Torrekens, S., Van-Leuven, F., and Delcour, A. 1998. Purification, properties and N-terminal amino acid sequence of a wheat gluten aspartic proteinase. *J. Cereal Sci.* 28:223-232.

Brettell, R. I. S., Chamberlain, D. A., Drew, A. M., McElroy, D., Witzens, B., and Dennis, E. S. 1994. Assessment of methods for the genetic transformation of wheat. Pages 3-9 in: *Improvement of Cereal Quality by Genetic Engineering*. Plenum Press: New York.

Field, J. M., Shewry, P. R., Burgess, S. R., Forde, J., Parmar, S. and Mifflin, B. J. 1983. The presence of high molecular weight aggregates in the protein bodies of developing endosperms of wheat and other cereals. *J. Cereal Sci.* 1:33.

Forde, J., Malpica, J.-M., Halford, N. G., Shewry, P. R., Anderson, O. D., Greene, F. C., and Mifflin, B. J. 1985. The nucleotide sequence of a HMW glutenin subunit gene from chromosome 1A of wheat (*Triticum aestivum* L.). *Nucleic Acids Res.* 13:6817-6832.

Halberd, N. P. 1985. Nucleotide sequence of a gene from chromosome 1D of wheat encoding a HMW-glutenin subunit. *Nucleic Acids Res.* 13:6833-6846.

Halford, N. G., Field, J. M., Blair, H., Urwin, P., Moore, K., Robert, L., Thompson, R., Flavell, R. B., Tatham, A. S., and Shewry, P. R. 1992. Analysis of HMW glutenin subunits encoded by chromosome 1A of bread wheat (*Triticum aestivum* L.) indicates quantitative effects on grain quality. *Theor. Appl. Genet.* 83:373-378.

Halford, N. G., Forde, J., Anderson, O. D., Greece, F. C., and Shewry, P. R. 1987. The nucleotide and deduced amino acid sequence of an HMW glutenin subunit gene from chromosome 1B of bread wheat (*Triticum aestivum* L.) and comparison with those of genes from chromosome 1A and 1D. *Theor. Appl. Genet.* 75:117-126.

Hirano, H. 1989. Microsequence analysis of winged bean seed proteins electroblotted from 2D-PAGE gel. *J. Protein Chem.* 8:115-130.

Hirano, H., and Watanabe, T. 1990. Microsequencing of proteins electrotransferred onto immobilizing matrices from polyacrylamide gel electrophoresis: Application to an insoluble protein. *Electrophoresis* 11:573-580.

Jackson, E. A., Holt, L. M., and Payne, P. I. 1983. Characterization of high molecular weight gliadin and low molecular weight glutenin subunits of wheat endosperm by 2D-PAGE electrophoresis and the chromosomal localization of their controlling genes. *Theor. Appl. Genet.* 66:29

Kasarda, D. D. 1989. Glutenin structure in relation to wheat quality. Pages 277-302 in: *Wheat is Unique*. Am. Assoc. Cereal Chem.: St. Paul, MN.

Kasarda, D. D., Bernardin, J. E., and Nimmo, C. C. 1976. Wheat proteins. Page 158 in: *Advances in Cereal Science and Technology*, Vol. 1. Am. Assoc. Cereal Chem.: St. Paul, MN.

Kasarda, D. D., Autran, J. C., Lew, E. J. L., Nimmo, C. C., and Shewry, P. R. 1983. N-terminal amino acid sequences ω-gliadins and ω-secalins implications for the evolution of prolamin genes. *Biochem. Biophys. Acta* 747:138-150.

Kasarda, D. D., Okita, T. W., Bernardin, J. E., Backer, P. A., Nimmo, C. C., Lew, E. J.-L., Dietler, M. D., and Greene, F. C. 1984. Nucleic acid and amino acid sequences of a-type gliadins from wheat (*Triticum aestivum*). *Proc. Natl. Acad. Sci. USA* 81:4712.

Lafiandra, D., and Kasarda, D. D. 1985. One- and two-dimensional polyacrylamide gel electrophoresis in a single gel: Separation of wheat proteins. *Cereal Chem.* 62:314-319.

Mackie, A. M., Sharp, P. J., and Lagudah, E. S. 1996. The nucleotide and derived amino acid sequence of a HMW glutenin gene from *Triticum tauschii* and comparison with those from the D genome of bread wheat. *J. Cereal Sci.* 24:73-78.

Masci, S., Egorov, T. A., Ronchi, C., Kuzmicky, D. D., Kasarda, D. D., and Lafiandra, D. 1999. Evidence for the presence of only one cysteine residue in the D-type low molecular weight subunits of wheat subunits of wheat glutenin. *J. Cereal Sci.* 29:17-25.

Nakamura, H. 1999. Identification of alleles for complex gene loci *Glu-A1*, *Glu-B1*, and *Glu-D1*, which code for high molecular weight subunits of glutenin in Japanese hexaploid wheat varieties. *J. Agric. Food Chem.* 47:5273-5277.

Nakamura, H., Sasaki, H., Hirano, H., and Yamashita, A. 1990. A high molecular weight subunit of wheat glutenin endosperm protein correlates with its flour quality. *Jpn. J. Breed.* 40:485-494.

Nakamura, H., Inazu, A., and Hirano, H. 1999. Allelic variation in high-molecular-weight glutenin subunit loci of *Glu-1* in Japanese common wheats. *Euphytica* 106:131-138.

O'Farrell, P. H. 1975. High resolution 2D-PAGE electrophoresis of proteins. *J. Biol. Chem.* 250:4007.

O'Farrell, P. Z., Goodman, H. M., and O'Farrell, P. H. 1977. High reso-

- lution 2D-PAGE electrophoresis of basic as well as acidic proteins. Cell 12:1133.
- Ovidio, D. R., Marchitelli, C., Cardelli, E., and Porceddu, E. 1999. Sequence similarity between allelic *Glu-B3* genes related to quality properties of durum wheat. Theor. Appl. Genet. 98:455-461.
- Payne, P. I., Corfield, K. G., and Blackman, J. A. 1979. Identification of a high-molecular-weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. Theor. Appl. Genet. 55:153-159.
- Payne, P. I., Holt, L. M., Jarvis, M. G., and Jackson, E. A. 1985. 2D-PAGE fractionation of the endosperm proteins of bread wheat (*Triticum aestivum*): Biochemical and genetic studies. Cereal Chem. 62:319-326.
- Rafalshi, J. A. 1986. Structure of wheat γ -gliadin gene. Gene 43:221-229.
- Reddy, P., and Apples, R. 1993. Analysis of a genomic DNA segment carrying the wheat high-molecular-weight (HMW) glutenin B \times 17 subunit and its use as an RFLP marker. Theor. Appl. Genet. 85:616-624.
- Shani, N., Steffen-Campbell, J. D., Anderson, O. D., Greene, F. C., and Galili, G. 1992. Role of the amino- and carboxy-terminal regions in the folding and oligomerization of wheat high-molecular weight glutenin subunits. Plant Physiol. 98:433-441.
- Shewry, P. R., Field, J. M., Faulks, A. J., Parmar, S., Mifflin, B. J., Dietler, M. D., Lew, E. J. L., and Kasarda, D. D. 1984. The purification and N-terminal amino acid sequence analysis of the high molecular weight glutenin polypeptides of wheat. Biochem. Biophys. Acta 788:23-34.
- Shewry, P. R., Halford, N. G., and Tatham, A. S. 1989. The high molecular weight subunits of wheat, barely and rye: genetics, molecular biology, chemistry and role in wheat gluten structure and functionality. Oxf. Surv. Plant Mol. Cell Biol. 6:163-219.
- Shewry, P. R., and Tatham, A. S. 1990. The prolamin storage proteins of cereal seeds: structure and evolution. Biochem. J. 267:1-12.
- Thompson, R. D., Bartels, D., Harberd, N. P., and Flavell, R. B. 1983. Characterization of the multigene family coding for HMW glutenin subunits in wheat using cDNA clones. Theor. Appl. Genet. 67:87.
- Vasil, V., Brown, S. M., Re, D., Fromm, M. E., and Vasil, I. K. 1991. Stably transformed callus lines from micro-projectile bombardment of cell suspension cultures of wheat. Biotechnology 9:743-747.

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