NOTE

Relationship of D-Zone Omega Gliadins to the Proteins Associated with Differences in Quality of Durum Wheats[1]

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Cereal Chem. 70(4):483–486

ABSTRACT

Gliadins from 40 durum wheats, representing a broad range of pasta quality, were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 7.5–9.0% gradient polyacrylamide gel system. Based on the presence or absence of the d_4 ω-gliadin component, durum genotypes could be divided into two groups similar to γ-42 and γ-45 or low molecular weight (LMW)-1 and LMW-2 types. The d_4 component was present in durum wheats with attributes of inferior quality and absent in wheats with attributes of superior quality. The ω-gliadin d_4, with a relative mobility of 33 and 38 in acid polyacylamide gel electrophoresis, was closely related to the presence of γ-gliadin 42 or LMW-1 subunits. The presence or absence of this specific protein component can be used in the prediction of quality for breeders' lines or commercial durum wheat samples using routine laboratory procedures and equipment.

Gliadin band γ-45, separated using acid-polyacrylamide gel electrophoresis (A-PAGE), is usually related to the superior viscoelastic properties of cooked pasta in durum wheats; γ-42 is associated with inferior quality (Damidaux et al 1978, Matsuo et al 1982, du Cros 1987). Kosmolak et al (1980), however, reported that some cultivars containing both 42 and 45 mobility bands had optimum, or less than optimum, viscoelastic properties. These bands were resolved using a unique system in which reproducibility is determined by the purity of chemicals and operator's skill. Moreover, A-PAGE requires special equipment designed or modified to accommodate quick polymerization. Handling of fragile (6% polyacrylamide) gels is tedious and demands special care. Quality and resolution of bands is poor and requires special processing (Lafiandra and Kasarda 1985, Branlard et al 1990, Lukow et al 1990). Solutions, buffers, and chemicals are entirely different than those used in commercially available sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) systems.

More recently, Khelifi et al (1992) and Branlard et al (1992) reported a two step A-PAGE/SDS-PAGE procedure to separate ω-gliadin. The procedure is sophisticated and labor-intensive and may not be suitable for routine use. Payne et al (1984) and Autran and Galterio (1989) associated some bands of ω-gliadins with a group of γ-gliadins, whereas Carrillo et al (1990) associated

[1]Contribution 1521 of Agriculture Canada Research Station, Winnipeg, Manitoba.

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low molecular weight (LMW) glutenin subunits LMW-2, LMW-2*, and LMW-2⁻ with γ-45; LMW-1 and LMW-1⁻ were associated with γ-42 gliadin. However, the distinction between LMW-1 (45.2 kDa) and LMW-2 (46 and 45 kDa) bands was not very clear. Despite such improvements in electrophoretic methodology, there is still a need for a more rapid procedure, particularly one less labor-intensive. The present study was carried out 1) to investigate proteins other than γ-gliadins and LMW glutenin subunits that can be used to predict the technological attributes of durum genotypes, and 2) to develop a simple one-dimensional, one-step procedure to resolve marker proteins.

MATERIALS AND METHODS

Plant Material

The 40 durum wheat cultivars and lines used in this study were obtained from N. K. Howes and D. Leisle, Agriculture Canada, Winnipeg Research Station (Table I). The hexaploid wheat cultivars (Courtot, Darius, Lancota, and Chinese Spring) used as reference for the D-zone gliadins, were obtained from G. Branlard, INRA, Clermont-Ferrand, France.

TABLE I
Distribution of the d_4 ω-Gliadin Component in Durum Wheats

Cultivars /Lines	Origin[a]	d_4 ω-Gliadin[b]	γ-Gliadin[c]	LMW Type[d]
Ardente	ITAL	o	45	2
Ambral	FR	o	45	2
Amarelrejo	PORT	o	45	2
Arcola	CDN	o	45	2
Attila	GER	o	45	2
Berillo	ITAL	o	42	2
Biodur	FR	o	45	2
Cando	USA	+	42	1
DT 367	CDN	o	45	2
DT 369	CDN	o	45	2
DT 430	CDN	o	45	2
DT 471	CDN	o	45	2
DT 474	CDN	o	45	2
DT 617	CDN	o	45	2
DT 624	CDN	o	45	2
Edmore	USA	o	45	2
Flodur	FR	o	45	2
Hercules	CDN	o	45	2
Kamilaroi	AUST	o	45	2
Leeds	USA	+	42	1
Maliani	ITAL	o	45	2
Medora	CDN	o	45	2
ND 86464	USA	o	45	2
Nugget	USA	+	42	1
Orgaz	PORT	o	45	2
Quilafen	CHIL	o	45	2
Ramsey	USA	+	42	1
Rugby	USA	+	42	1
Sentry	USA	+	42	1
Sceptre	CDN	o	45	2
Stewart	USA	+	42	1
Taganrog	ARG	o	45	2
Valnova	ITAL	o	45	2
Vic	USA	o	45	2
VIR 51658	CIS	o	45	2
Wakooma	CDN	o	45	2
Ward	USA	+	42	1
Wascana	CDN	+	42	1
Westbred	USA	o	45	2
Yavaros	CIS	o	45	2

[a] ITAL = Italy, FR = France, PORT = Portugal, CDN = Canada, GER = Germany, USA = United States of America, AUST = Australia, CHIL = Chile, ARG = Argentina, CIS = Commonwealth of Independent States.
[b] + Present, o absent.
[c] Predominantly stained gliadin band.
[d] Low molecular weight glutenin subunits.

Preparation of Protein Solutions

The distal half-grain containing endosperm was crushed between smooth ends of a pair of pliers and soaked overnight in five volumes of 70% ethanol at room temperature (21 ± 2°C). The following morning, the seed meal-ethanol mixture was homogenized using an Eppendorf tissue grinder. The clear supernatant was obtained by centrifugation at approximately 12,700 × *g* for 5 min at room temperature. It was then mixed 1:1 with the modified dye solution C. To prepare modified solutions, 5 ml of solution C (Singh et al 1991) was freshly supplemented with 0.05 g of dithiothreitol and 70 μl of 4-vinylpyridine solution before mixing with the ethanol extract. Solution C contained 2% SDS, 40% glycerol, and 0.02% bromophenol blue in 0.08*M* Tris HCl, pH 8.0. The mixture was incubated at 65°C for 30 min and centrifuged at 12,700 × *g* for 2 min. Alkylation of proteins with 4-vinylpyridine was necessary to prevent reoxidation during analyses and storage (Huebner and Bietz 1987). Electrophoresis slots were loaded with the protein solution (8 μl). The residue produced after the first centrifugation can be used for analysis of glutenin if required.

For A-PAGE, the protein solution was prepared according to Sapirstein and Bushuk (1985) by mixing the ethanol extract with an equal volume of methyl green dye solution prepared in aluminum lactate (pH 3.1).

Electrophoresis

SDS-PAGE was carried out in a 7.5–9.0% gradient polyacrylamide gel with a 2.5-cm stacking gel of 3% polyacrylamide at a constant current of 40 mA per gel for 3–3.5 hr at 25°C using a vertical slab gel unit (Hoefer Scientific Instrument, San Francisco, CA). Gels stained overnight with Coomassie brilliant blue (CBB) R-250 were destained with water, and the intense background was further clarified by CBB-G-250 staining solution (Blakesley and Boenzi 1977). A tissue paper was floated over the gel to adsorb excess stain during the destaining process.

For A-PAGE, 10% glycerol was added to the gel solution to improve band quality (Hussain and Bushuk 1991). Other electrophoresis conditions were the same as described by Sapirstein and Bushuk (1985). A locally constructed electrophoresis unit (Hussain et al 1988) was used to cast and run acrylamide gel electrophoresis in 3-mm gel.

Uniformity of the electrophoretic band patterns was checked using 10 randomly selected seeds of cultivars. SDS-PAGE patterns of the 10 seeds were identical. All SDS-PAGE and A-PAGE results were reproducible when retested using selected genotypes. All extracts were analyzed within two to three days of extraction. Storage for five to six days, even at freezing temperature (−15°C), caused a decrease in band intensity (results not shown).

Nomenclature

Nomenclature proposed by Branlard et al (1992) and Khelifi et al (1992) was used to identify D-zone gliadin bands separated in SDS-PAGE. Bands separated in A-PAGE were labeled according to the nomenclature of Bushuk and Zillman (1978) and Bushuk and Sapirstein (1990).

RESULTS AND DISCUSSION

The ethanol-extractable durum proteins were resolved into a distinct mobility region consisting of one to three intensely stained bands at approximately 65 kDa, two of which corresponded to the d_4 and d_5 ω-gliadin of the reference hexaploid wheat cultivars, Darius and Chinese Spring (Fig. 1). The d_5 gliadin was present in all 40 durum cultivars and lines except Arcola, DT 430, DT 471, DT 474, Hercules, and Medora, where a corresponding band had a slightly faster mobility. We designated this band d_{5*}. Another intensely stained band corresponding to d_4 was present in nine durum samples (Table I). Based on the presence or absence of d_4 polypeptide, the tested durum collection can be divided into two groups. Electrophoresis results revealed that the cultivars (lines) with γ-42 contained d_4 ω-gliadin band. Consequently, it appears that the presence of d_4 gliadin band (arrowed in Fig.

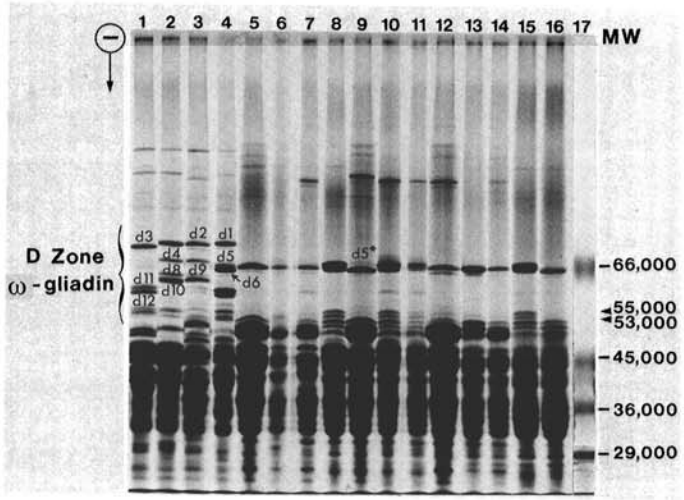

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis separation of the ethanol-extractable proteins of the distal halves of single wheat kernels in a 7.5–9.0% gradient gel showing D-zone ω-gliadins. Lanes 1–17: Courtot, Darius, Lancota, Chinese Spring, Vic, Amerelejo, Kamilaroi, Rugby, Hercules, Leeds, Sentry, Ambral, Westbred, Biodur, Stewart, Quilafen, and molecular weight (MW) marker proteins (bovine albumin, 66 kDa; egg albumin, 45 kDa; glyceraldehyde-3-phosphate, 36 kDa, and carbonic anhydrase, 29 kDa). Lanes 1-4 contain hexaploid cultivars used as standards for the D-zone ω-gliadins.

1) is associated with the poor quality attributes. Contrary to the functional relationship between gliadin patterns and end-use quality, the Berillo cultivar possesses a γ-42 gliadin but has superior quality. The absence of the d_4 protein band in Berillo is a more accurate indicator of its quality.

Examination of the electrophoretic patterns of alcohol-soluble proteins (Fig. 1) revealed two additional bands (53 and 55 kDa) in the patterns of durum wheats containing d_4 ω-gliadin. These proteins warrant further investigation into their association with end-use quality.

The d_5 and d_{4+5} bands were eluted from SDS-PAGE electrophoregrams of Quilafen and Stewart, respectively, in a minimum volume (200 μl) of 70% ethanol by crushing the unstained gel strips (10 × 0.5 cm) corresponding to the stained position of d_5 and d_{4+5}. The ethanol extract (25 μl) was mixed 1:1 (v/v) with the dye solution and separated in an A-PAGE system (Fig. 2). Apparent dissociation of some of the proteins from SDS in ethanol and aluminum lactate buffer permitted their resolution in A-PAGE. Bands d_4 and d_5 did not correspond to γ-gliadins 42 and 45 but appeared in the boundaries of ω-gliadins. Band d_5 appeared at relative mobility 35 and d_4 appeared at relative mobility 33 and 38. The d_4 and d_5 gliadins were also detectable when the protein solution prepared for SDS-PAGE (containing SDS, dithiothreitol, and 4-vinylpyridine) was separated in polyacrylamide gel at pH 3.1 (lanes 5 and 6, Fig. 2). These bands appear to correlate with those seen in the corresponding A-PAGE extracts (lanes 1 and 2, Fig. 2) and those eluted as d_4 and d_5 (lanes 3 and 4, Fig. 2). It appears that the mobility of d_4 and d_5 polypeptides was not affected by SDS, pH, reduction, or alkylation treatments. The fact that the two ω-gliadins remain separated in both A-PAGE and SDS-PAGE systems implies that these two monomers may differ in size and charge. Based on differences in mobility in acid and SDS polyacrylamide gels, d_4 appeared to be a larger molecule than d_5 with relatively more positive net charge.

The procedure used for resolving D-zone proteins varied substantially from that reported by Branlard et al (1992) and Khelifi et al (1992). Our one-step, one-dimensional procedure gave patterns comparable to those obtained by the complex two-step procedure. Resolution and quality of the D-zone protein bands was superior in the 7.5–9% gradient polyacrylamide gel compared with the separation in the linear gel. The extraction procedure was effective both for coarsely crushed half-seed and for

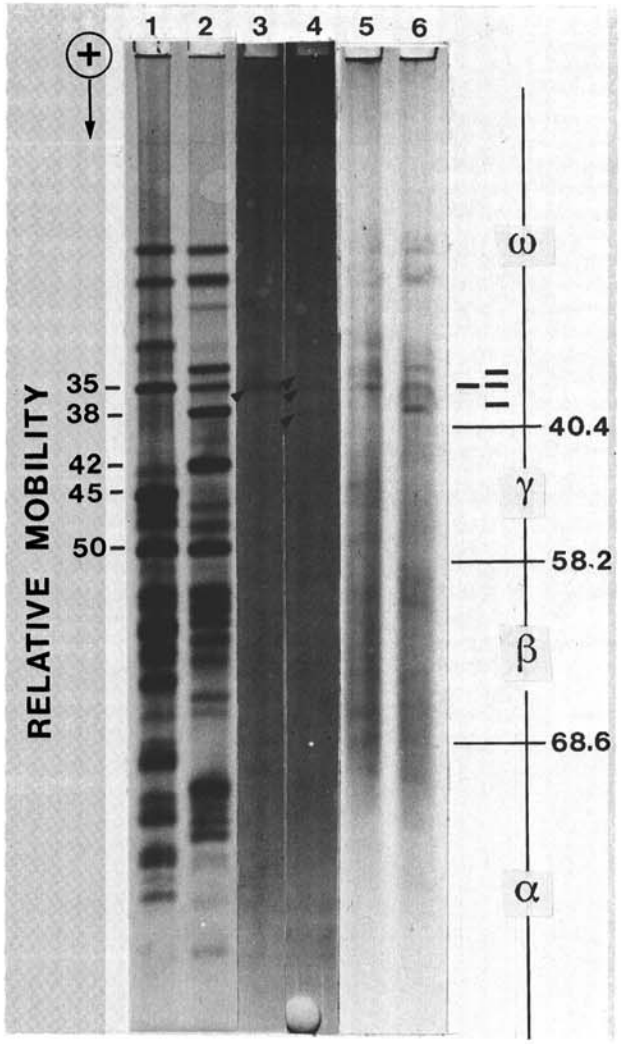

Fig. 2. Acid-polyacrylamide gel electrophoresis separation of various protein solutions of cultivars Quilafen (lanes 1, 3, and 5) and Stewart (lanes 2, 4, and 6). Lanes 1 and 2: ethanol extract. Lanes 3 and 4: d_5 and d_{4+5} ω-gliadin bands eluted from the sodium dodecyl sulfate gel. Lanes 5 and 6: protein solution prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Arrows = gliadin bands 33, 35, and 38. Classes of gliadins represented by Greek letters.

commercially milled samples.

In conclusion, the presence or absence of ω-gliadin d_4 can be used as a means of predicting end-use quality of durum wheat. These proteins are easier to extract and can be visualized more clearly as components of a distinct zone in an SDS-PAGE system than they can as LMW glutenin subunits and γ-gliadins in an A-PAGE system. The procedure is convenient and provides a good resolution of the specific proteins. Since the end-use quality seems to be indirectly related to the presence or absence of d_4 gliadin, production of a monoclonal antibody specific to this particular polypeptide would facilitate early screening of breeders' lines for quality. However, the usefulness of these ω-gliadin indicators in a breeding situation remains to be proven.

ACKNOWLEDGMENTS

We thank D. Leisle, N. Howes, and G. Branlard for the seed supply, R. Sims for graphics, and the Western Grains Research Council for financial assistance.

LITERATURE CITED

AUTRAN, J. C., and GALTERIO, G. 1989. Associations between electrophoretic composition of proteins, quality characteristics and agronomic attributes of durum wheats. I. Protein-protein associations. J. Cereal Sci. 9:179-194.

BLAKESLEY, R. W., and BOENZI, J. A. 1977. A new staining technique for proteins in polyacrylamide gels using Coomassie Brilliant Blue G 250. Anal. Biochem. 82:580-582.

BRANLARD, G., PICARD, B., and COURVOISIER, C. 1990. Electrophoresis of gliadins in long acrylamide gels: Method and nomenclature. Electrophoresis 11:310-314.

BRANLARD, G., KHELIFI, D., and LOOKHART, G. 1992. Identification of some proteins of wheat separated by a two-step acid polyacrylamide gel electrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis technique. Cereal Chem. 69:677-678.

BUSHUK, W., and SAPIRSTEIN, H. D. 1990. Modified nomenclature for gliadins. Pages 454-458 in: Gluten Proteins. W. Bushuk and R. Tkachuk, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

BUSHUK, W., and ZILLMAN, R. R. 1978. Wheat cultivar identification by gliadin electrophoregrams I. Apparatus, method and nomenclature. Can. J. Plant Sci. 58:505-515.

CARRILLO, J. M., VAZQUEZ, J. F., and ORELLANA, J. 1990. Relationship between gluten strength and glutenin proteins in durum wheat cultivars. Plant Breeding 104:325-333.

DAMIDAUX, R., AUTRAN, J. C., GRIGNAC, P., and FEILLET, P. 1978. Mise en evidence de relations applicables en selection entre l'electrophoregramme des gliadines et les proprietes viscoelastiques du gluten de *Triticum durum*. Desf. C. R. Acad. Sci. Ser. D 287:701-704.

du CROS, D. L. 1987. Glutenin proteins and gluten strength in durum wheat. J. Cereal Sci. 5:3-12.

HUEBNER, F. R., and BIETZ, J. A. 1987. Improvements in wheat protein analysis and quality prediction by reversed-phase high-performance liquid chromatography. Cereal Chem. 64:15-20.

HUSSAIN, A., and BUSHUK, W. 1991. Changes in protein electrophoretic patterns during germination of broad beans (*Vicia faba* L.) with and without hard-to-cook characteristics. Euphytica 55:131-139.

HUSSAIN, A., BUSHUK, W., RAMIREZ, H., and ROCA, W. M. 1988. A practical guide for electrophoretic analysis of isoenzymes and proteins in cassava, field beans and forage legumes. CIAT: Cali, Colombia.

KHELIFI, D., BRANLARD, G., and BOURGOIN-GRENECHE, M. 1992. Diversity of some D-zone omega gliadins of bread wheat as revealed by 2 step A-PAGE/SDS-PAGE technique. J. Genet. Breed. 46:351-358.

KOSMOLAK, F. G., DEXTER, J. E., MATSUO, R. R., LEISLE, D., and MARCHYLO, B. A. 1980. A relationship between durum wheat quality and gliadin electrophoregrams. Can. J. Plant Sci. 60:427-432.

LAFIANDRA, D., and KASARDA, D. D. 1985. One- and two-dimensional (two-pH) polyacrylamide gel electrophoresis in a single gel: Separation of wheat proteins. Cereal Chem. 62:314-319.

LUKOW, O. M., ZHANG, H., and CZARNECKI, E. 1990. Milling, rheological, and end-use quality of Chinese and Canadian spring wheat cultivars. Cereal Chem. 67:170-176.

MATSUO, R. R., DEXTER, J. E., KOSMOLAK, F. G., and LEISLE, D. 1982. Statistical evaluation of tests for assessing spaghetti-making quality of durum wheat. Cereal Chem. 59:222-228.

PAYNE, P. I., JACKSON, E. A., and HOLT, L. M. 1984. The association between γ-gliadin 45 and gluten strength in durum wheat varieties: A direct causal effect or the result of genetic linkage? J. Cereal Sci. 2:73-81.

SAPIRSTEIN, H. D., and BUSHUK, W. 1985. Computer-aided analysis of gliadin electrophoregrams. I. Improvement of precision of relative mobility determination by using a three reference band standardization. Cereal Chem. 62:372-377.

SINGH, N. K., SHEPHERD, K. W., and CORNISH, G. B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J. Cereal Sci. 14:203-208.

[Received December 7, 1992. Accepted May 4, 1993.]