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Genetic Control of Endosperm Proteins in Wheat

1. The Use of High Resolution One-dimensional Gel Electrophoresis for the Allocation of Genes Coding for Endosperm Protein Subunits in the Common Wheat Cultivar Chinese Spring

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Summary. Total endosperm protein subunits, extracted from the common wheat cultivar Chinese Spring and from some of its aneuploid lines, were fractionated according to their molecular weight (MW) in an improved high resolution one-dimensional sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE). The resolution obtained by this method and, in particular, that of the high molecular weight (HMW) glutenin and gliadin subunits approached that of a previous report in which two-dimensional fractionation system based on charge and MW was used. In the cultivar Chinese Spring, 21 discrete protein bands were resolved and the chromosomes controlling many of them were either reconfirmed, or, in some cases, established. The advantages of this high resolution SDS PAGE technique are discussed.

Key words: Common wheat – *Triticum aestivum* – Electrophoresis – Endosperm proteins

Introduction

The production of aneuploid lines in the common wheat cultivar Chinese Spring (Sears 1954, 1966) has opened new possibilities for studying the genetic control of many morphological, physiological and biochemical traits in this important crop plant. In recent years, by the use of these aneuploid lines, the genetic control of wheat endosperm proteins has been extensively investigated and many genes coding for specific protein subunits were allocated to specific chromosomes. Moreover, the nutritional value of various endosperm proteins as well as their role in bread making have been determined (for review, see Kasarda et al. 1976a; Konzak 1977; Wall 1979).

Fractionation of endosperm proteins from these aneuploid lines, which were previously carried out by various systems of one-dimensional gel electrophoresis, enabled the resolution of only a few gliadin (Shepherd 1968; Kasarda et al. 1976b) and glutenin subunits (Bietz et al. 1975; Khan and Bushuk 1977; Lawrence and Shepherd 1980). In other studies, by employing different two-dimensional gel electrophoresis systems, Wrigley and Shepherd (1973) resolved as many as 46 gliadin bands while Brown et al. (1979) resolved over 20 bands from the total endosperm protein subunits of the cultivar Chinese Spring. The genes controlling most of these bands were allocated to chromosomes of homoeologous groups 1 and 6. Although two-dimensional polyacrylamide gel electrophoresis (PAGE) systems may achieve a high resolution, they have two main limitations: first, an accurate quantitative estimation of proteins is rather difficult to obtain and secondly, only one sample per slab gel can be applied as compared to about 25 samples in the one-dimensional PAGE systems. These limitations render the two-dimensional PAGE systems less suitable for a large scale genetic study, which often requires screening of many samples. In addition, the comparison and fine quantitative analysis of similar protein bands from different samples is quite inaccurate and hence very limited.

A one-dimensional high resolution sodium dodecyl sulphate (SDS) PAGE as described in this paper is designed to overcome these limitations. This technique, which is a modification of that described by Laemmli (1970), was used to reevaluate previous data obtained from two-dimensional gel electrophoresis on the chromosomal control of endosperm protein subunits in the cultivar Chinese Spring. In addition, the chromosomal control of additional protein bands, as established by this technique, is reported.

Materials and Methods

Plant Material

Seeds of the cultivar Chinese Spring of common wheat, *Triticum aestivum* L. em Thell. and most of its aneuploid lines were kindly provided by E.R. Sears. The aneuploid lines included: 1) compensating nullisomic-tetrasomic lines of homoeologous groups 1 and 6, i.e., lines deficient for one pair of homologous chromosomes but having four doses of another chromosome of the same homoeologous group; 2) ditelosomic lines of homoeologous group 1, i.e., lines deficient for one pair of chromosome arms. The ditelosomic line for the short arm of chromosome 1D (1DS) is not available.

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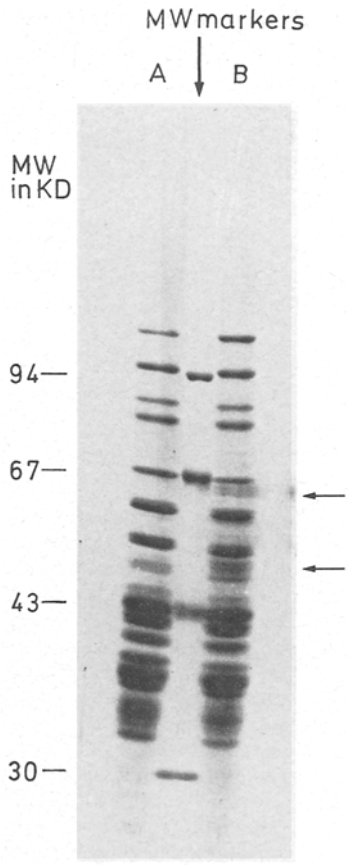

Fig. 1. SDS PAGE migration pattern of total endosperm protein subunits extracted from common wheat cultivar Chinese Spring by (*A*) Al lactate followed by sample buffer (see Materials and Methods) and (*B*) directly by sample buffer. The middle lane contains MW markers with size indicated on the left

Extraction of Endosperm Proteins

Embryo-less grains were ground by a pestle and mortar and homogenated in a solution consisting of 8 mM aluminum lactate (ICN) and 10% glycerol (BDH), pH 3.2, at a concentration of 20 mg/ml. The homogenate was stored at –40 °C until used. Prior to electrophoresis the homogenate was defrosted and well mixed, after which 30 µl were drawn into a 1.5 ml eppendorf tube containing 20 µl of a solution consisting of 15% 2-mercapto-ethanol (2-ME), 10% glycerol, 9% SDS (BDH), 0.1% bromophenolblue, 187.5 mM of Tris-HCl (Sigma) pH 6.8 (3× sample buffer) and 7 µl of 0.3 M NaOH. This mixture was allowed to stay at room temperature for two hours. Following that, it was brought to 100 °C for two min, centrifuged in an eppendorf centrifuge for two min and a sample of 15–25 µl of the supernatant loaded directly on each lane of the gel.

Regarding protein solubilization, this method was identical to a control treatment in which grains were homogenized directly in 1× sample buffer (Fig. 1), except for two diffused bands which appeared inconsistently in the control and were not observed in the material homogenated in Al lactate (Fig. 1 b, marked by arrows). These bands were more intense after a prolonged storage of the proteins in 1× sample buffer and may represent proteolytic activity or subunits reassociation. The possibility that these bands are intact subunits which are soluble only in the control extraction cannot be ruled out but seems unlikely since the SDS + 2–ME containing solution used in both treatments is known to solubilize more than 95% of the endosperm proteins in wheat (Bietz et al. 1975). However, the Al lactate homogenization technique offers two main advantages: 1) The Al lactate homogenate can be stored at –40 °C for at least six months without any detectable changes in the protein pattern in contrast to a prolonged storage in 1× sample buffer which causes subunit instability (Galili unpublished); 2) Multimeric proteins, stored in the Al lactate buffer, can be freshly reduced to their subunits just prior to electrophoresis, thus preventing problems of subunits reassociation and eliminating the need of chemical blocking of S-H groups.

Preparation of Polyacrylamide Gels

Endosperm protein subunits were fractionated in 15 cm wide, 0.16 cm thick and 13 cm long slab gels (except for Fig. 3 in which 18 cm long gels were used) in a modified discontinuous SDS PAGE of Laemmli (1970). The gels were composed of two layers: the lower one was a gradient gel and the upper a stacking gel. The gradient gel consisted of 0.375 M Tris-HCl, 0.1% SDS pH 8.8 and a gradient of acrylamide (BDH), bisacrylamide (Eastman kodak) and glycerol ranging from 7%, 0.187% and 1% to 12%, 0.32% and 4.5%, respectively. This gel was polymerized by the addition of 7 µl N,N,N′,N′ – tetramethylethylenediamine (TEMED) (Eastman kodak) and 70 µl of 10% ammonium persulphate (APS) (BDH) per 20 ml of gel solution.

The stacking gel consisted of 5% acrylamide, 0.13% bisacrylamide, 0.1% SDS, 0.125 M Tris-HCl pH 6.8 and was polymerized by the addition of 8 µl TEMED and 24 µl APS per 8 ml solution.

The regular gels (13 cm long) were run for 4 h at a constant of 150 volts while the long gels (18 cm) were run for 17 h at a constant of 100 volts. The electrode buffer consisted of 0.025 M Tris-glycine, 0.1% SDS pH 8.5.

Table 1. Effect of genetic deficiency on the appearance of specific bands in the cultivar Chinese Spring of common wheat

Deficient chromosome arm or chromosome	Bands showing effect[a]		Reference
	Absence	Reduced staining	
1BL	2, 3	–	Fig. 2 d – f; Fig. 3 d – e.
1BS	5, 6, 7, 12	13, 14, 19	Fig. 2 c, e, f; Fig. 3 d – e.
1DL	1, 4	–	Fig. 2 a, b; Fig. 3 f – g.
1DS	8, 9, 10	17	The line DT1Ds is not shown
1AL	–	–	Fig. 2 h – j; Fig. 3 a – b.
1AS	21	13, 15	Fig. 2 g, i, j; Fig. 3 a – b.
6B	16	–	not shown

[a] Band numbers are as indicated in Fig. 4 and at the right of Fig. 2

Staining and Destaining

At the termination of the runs the gels were soaked twice in an excess of 10% methanol for 30 min and then stained for 16 h in 1% coomassie brilliant blue R-250 (Sigma) dissolved in absolute ethanol and diluted 1 : 20 in 12.5% trichloroacetic acid (TCA). Gels were destained for 4 h in 12.5% TCA until the background became clear and then transferred into distilled water.

Estimation of MW

The MW of the various endosperm protein subunits was estimated by comparison with protein markers of known MW (Pharmacia) which were fractionated in a parallel lane. The protein markers were phosphorilase-B [94 kilo dalton (KD)], bovine serum albumin (67 KD), ovalbumin (43 KD) and carbonic anhydrase (30 KD).

Results and Discussion

Total endosperm proteins were extracted from single embryo-less grains of euploid and various aneuploid lines of the cultivar Chinese Spring and fractionated in one-dimensional SDS PAGE. This SDS PAGE has been proven to be of higher resolution than any other one-dimensional SDS PAGE systems previously used for wheat endosperm proteins. Twenty-one discrete bands were resolved in the euploid line (Fig. 2k; see band no. on the right). Three main sections could be detected in each gel, i.e., bands 1–4 of HMW glutenins (Bietz et al. 1975), bands 5–9 of HMW gliadins (probably identical to ω gliadins, Bietz et al. 1977) and bands 10–21 representing subunits of lower molecular weight.

Although bands 6 and 7, as well as 8 and 9 were poorly resolved in these gels, their appearance as four discrete different bands was highly reproducible. These bands were better resolved in the longer (18 cm) gels (Fig. 3, bands 6–9).

The chromosomal control of specific bands was inferred from their absence or reduced staining in various aneuploid lines deficient for a given chromosome or chromosome arm. These results are summarized in Table 1. All the subunits in the fractions of HMW glutenins (bands 1–4) and HMW gliadins (bands 5–9) as well as several subunits of lower MW were found to be controlled by chromosomes of homoeologous group 1 as shown in Fig. 4 which illustrates a diagram of a typical SDS PAGE pattern of the cultivar Chinese Spring. Band 16 was found to be solely controlled by chromosome 6B (data not shown). A faint HMW glutenin band was detected in the aneuploid lines lacking chromosome 1D (Fig. 2a, b; below band

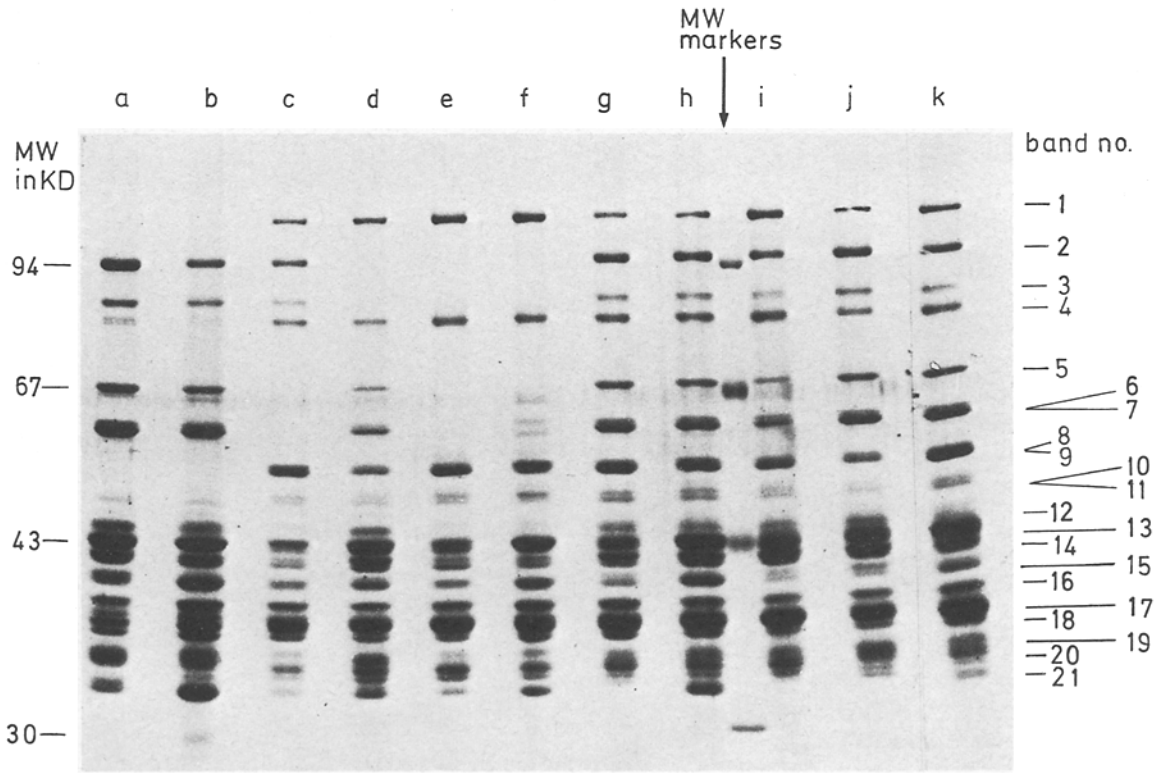

Fig. 2. SDS PAGE migration pattern of total endosperm protein subunits extracted from various aneuploid lines of common wheat cultivar Chinese Spring. (*a*) nullisome 1D tetrasome 1B (N1DT1B); (*b*) N1DT1A; (*c*) ditelosome 1B long (DT1BL); (*d*) DT1BS; (*e*) N1BT1D; (*f*) N1BT1A; (*g*) DT1AL; (*h*) DT1AS; (*i*) N1AT1D; (*j*) N1AT1B; (*k*) Chinese Spring. *MW* markers are included with *MW* indicated on the left

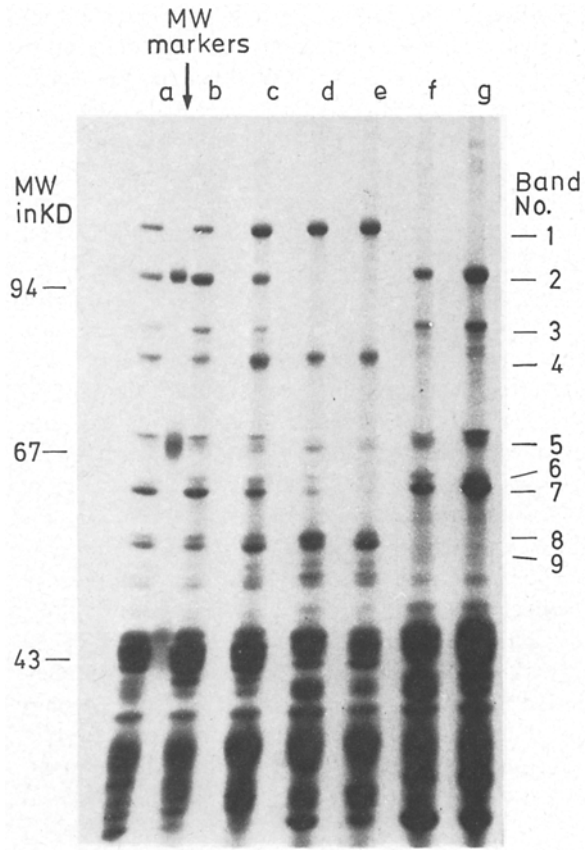

Fig. 3. Fractionation of total endosperm protein subunits extracted from various aneuploid lines of common wheat cultivar Chinese Spring, on 18 cm long SDS PAGE. (*a*) Chinese Spring; (*b*) N1AT1B; (*c*) N1AT1D; (*d*) N1BT1A; (*e*) N1BT1D; (*f*) N1DT1A; (*g*) N1DT1B. *MW* markers are included with *MW* indicated on the left

no. 3) which was not detected in the euploid (Fig. 2k). This band which is slightly heavier and thus migrates somewhat slower than band 4 is more intense in the line nullisomic for chromosome 1D and tetrasomic for 1B (N1DT1B) (Fig. 2a) than in N1DT1A (Fig. 2b). Thus, this band is probably controlled by chromosome 1B. The ditelosomic line for chromosome 1AS, i.e., the line deficient for the pair of chromosome arms 1AL, was identical to the euploid line (compare lanes h and k in Fig. 2), indicating that there is no detectable band in the cultivar Chinese Spring which is controlled by this chromosome arm. Among the 21 bands which were detected in the cultivar Chinese Spring, 13 bands were exclusively controlled by a specific chromosome (Fig. 4, bands 1–10, 12, 16 and 21) while five were heterogenous bands each composed of several subunits and controlled by several chromosomes (no. 13–15, 17 and 19). The chromosomal control of the remaining three bands (11, 18 and 20) could not be determined from this PAGE. In addition, the results show (Fig. 4) that out of the 17 subunits which were allocated to chromosomes in the cultivar Chinese Spring, 10 are controlled by chromosomes of genome B, six by genome D and only three subunits by genome A. This indicates that a non-random process of diploidization of endosperm protein genes has occurred during the course of evolution of common wheat. It is also shown (Fig. 4) that while the HMW glutenins are controlled by the long arms of homoeologous group 1, all the rest of the subunits of this homoeologous group are controlled by its short arm. The results obtained by the high resolution one-dimensional SDS PAGE which was used in this work are in accordance with the previous, more limited, results obtained from various techniques of one-dimensional SDS PAGE (Bietz et al. 1975; Khan and Bushuk 1977; Lawrence and Shepherd 1980). In addition, the results of this report and those obtained by the two-dimensional PAGE system of Brown et al. (1979) (Table 2) are in good agreement. However, the chromosomal control of some partially resolved bands (bands no. 13, 14, 18 and 19) as well as one discrete band (band no. 21) was not previously reported.

The high resolution of the one-dimensional system described in this paper offers three main advantages over the two-dimensional PAGE system of Brown et al. (1979): 1) This procedure is much shorter and simpler to perform than the two-dimensional system; 2) As

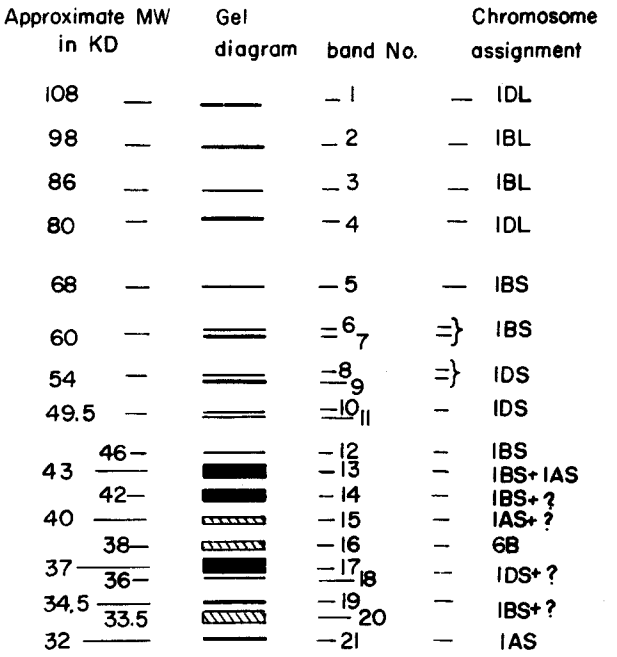

Fig. 4. Diagram of the SDS PAGE fractionation pattern of total endosperm protein extracted from common wheat cultivar Chinese Spring and corresponding to Fig. 2. The width and intensity of the various bands are expressed by the area and darkness of each band

Table 2. Comparison of the subunits which were allocated to chromosomes by Brown et al. (1979) using two-dimensional PAGE with the subunits reported in this paper using one-dimensional PAGE

Chromosome or chromosome arm location of the gene	Subunit reported in this paper		Corresponding subunit reported by Brown et al. 1979	
	Subunit no.	MW in KD	Subunit no.	MW in KD
1DL	1	108	1 (5 spots)	125
	4	80	2 (2 spots)	88
1BL	2	98	–	–
	3	86	–	–
1AS	13[a]	43	10	50
	21	32	–	–
1BS	5	68	3	70
	–	–	3a	71
	6	60.5	4	63
	7	59.5	5	64
	12	46	12	52
	13[a]	43	–	–
	14[a]	42	–	–
	19[a]	34.5	–	–
1DS	8	54.5	8[b]	62
	9	53.5	7[b]	60
	10	50	9[b]	56
	18[a]	36	–	–
	–	–	11	53
1B	–	–	6a	55
1D	–	–	6b	55
	–	–	6c	55

[a] Partially resolved band

[b] The exact correspondence of specific subunits controlled by chromosome 1D and chromosome arm 1DS of Brown et al. (1979) to subunits resolved in this report can not be determined

many as twenty-five samples can be loaded on each gel as compared with a single sample in the two-dimensional system; 3) This procedure offers a fine quantitative analysis of the resolved protein bands which has been proven useful in further studies on the mode of expression of some of these bands (Galili and Feldman in preparation).

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