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# Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutelin *

## 1. Variation and genetic control of the subunits in hexaploid wheats

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**Summary.** A collection of 222 hexaploid wheat cultivars (including the 207 cultivars studied by Gupta and Shepherd in 1988) from 32 countries was analyzed for variation in the banding patterns of LMW subunits of glutenin using a modified two-step 1-D SDS-PAGE. Seventy percent ethanol at high temperature ($\geq 50\,^{\circ}C$) was used to selectively dissolve the native glutenins containing A, B, and C subunits and not the albumins and globulins (non-prolamins). This procedure allowed the glutenin subunits A, B and C to be separated in a background free of albumins and globulins, which normally overlap the B and C subunits (LMW subunits of glutenin). Although 40 different B and C subunits were detected, except where the cultivars carried a *1BL-1RS* translocation or *1B/1R* substitution, each cultivar exhibited from 7 to 16 subunits. These subunits could be divided into 20 band patterns which fell into three groups on the basis of their mutual exclusiveness, with 6, 9, and 5 patterns. Analysis of substitution lines revealed that the different patterns in these groups are controlled by genes on chromosomes *1A*, *1B*, and *1D*, respectively. The least number of subunits was controlled by chromosome *1A* and approximately 40% of the cultivars did not contain any band controlled by this chromosome. Thirteen of the cultivars were found to consist of two biotypes with respect to LMW subunits of glutenin. The genetic, evolutionary, and technological implications of these findings are discussed.

**Key words:** LMW subunits of glutenin – Bread wheat – Allelic variation – Chromosomal location – Substitution lines

* The term 'glutelin' refers to the polymeric prolamins of cereals and, in the case of wheat, it is called glutenin

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### Introduction

During the last 20 years, there have been significant developments in the genetic characterization of the gluten proteins in hexaploid wheats, by utilizing improved methods of protein fractionation and the appropriate genetic stocks (see Shepherd 1988, for a review). The gliadins and HMW subunits of glutenin are easily resolved by 1-D SDS-PAGE and exhibit extensive pattern variation among different cultivars. The availability of several sets of intervarietal substitution lines, in which individual chromosome pairs of a recipient variety are substituted by the homologous chromosome pairs from another variety, has enabled the genes controlling pattern variation in gliadins and HMW subunits (A subunits) of glutenin to be assigned to particular chromosomes in several cultivars. The HMW subunits of glutenin have been shown to be controlled by genes (*Glu-1*) located on the long arms of group 1 chromosomes, whereas gliadins are controlled by genes (*Gli-1*, *Gli-2*) located on the short arms of both group 1 and group 6 chromosomes (Shepherd 1988).

In contrast to these two groups of proteins, the LMW subunits (B and C subunits) of glutenin have proved much more difficult to analyze in a 1-D electrophoretic system, mainly because of their overlapping mobilities with the gliadins. Previous studies by Jackson et al. (1983) used 2-D electrophoresis and located the genes controlling most of the LMW subunits on the short arms of group 1 chromosomes in Chinese Spring wheat. Payne et al. (1984a), using 1-D SDS-PAGE, were able to map the genes controlling some easily recognizable B subunits at the *Gli-1* loci. These separation techniques, however, lacked general application for studying the variability in B and C subunits and their inheritance, because the two-dimensional systems could analyze only one or two sam-

ples per gel and, furthermore, these subunits cannot be directly distinguished from gliadins. Consequently, only a few B subunit differences had been detected in both durum and bread wheats (Payne et al. 1984a, 1984b). The introduction of a two-step, 1-D SDS-PAGE procedure by Singh and Shepherd (1985, 1988), however, provided a rapid method for analyzing a large number of samples in a single gel in a gliadin-free background. Using this method, Singh and Shepherd (1988) were able to map the genes controlling some of these subunits at the *Glu-3* loci, and further investigations revealed extensive variation for these subunits in durum and bread wheats (Gupta and Shepherd 1987, 1988).

This two-step method, however, also separated some globulins (Singh and Shepherd 1985) and albumins (Gupta and Shepherd 1987) with similar mobility to the B and C subunits of glutenin. The procedure has now been modified to allow the latter subunits to be analyzed in a background free from albumins and globulins. The data obtained from analysis of the pattern variation in 222 hexaploid wheat cultivars (including the 207 cultivars previously analyzed by Gupta and Shepherd 1988) and their chromosomal controls as determined by analyzing various substitution lines are described.

## Materials and methods

*Genotypes analyzed*

Two hundred and twenty-two lines of hexaploid wheat (including cultivars and some advanced generation breeding lines) from 32 countries were analyzed. Details of these lines along with their country of origin have been listed elsewhere (Gupta 1989) and are available on request to R.B. Gupta. Seed samples of some of these cultivars were kindly provided by Mr. M. Mackey (Australian Wheat Collection, Tamworth) and others were taken from Waite Agricultural Research Institute stocks.

The intervarietal group 1 chromosome substitution lines of Cheyenne (Morris et al. 1966), Capelle-Desprez (Law et al. 1978), Thatcher, Kenya Farmer, Hope, and Timstein (Sears et al. 1957) in Chinese Spring were analyzed to determine the chromosomal control of the LMW subunits of glutenin in these cultivars. The following intergeneric substitution lines were also analyzed to determine the chromosomal control of these subunits in the recipient wheat cultivars: Rescue × *Elytrigia elongata* substitution (*1A*) and (*1D*) (Hurd 1959) and Kharkov × Dakold rye substitution *3R* (*1A*), *3R* (*1B*) (Lee et al. 1969). Seed samples of the substitution lines involving Capelle-Desprez, Kenya Farmer, and Thatcher were kindly provided by Dr. R.A. McIntosh (University of Sydney). The others came from stocks maintained at the Waite Agricultural Research Institute.

*Extraction of endosperm proteins*

The total unreduced proteins were extracted in TRIS-HCl buffer (pH 6.8) containing 4% SDS following Singh and Shepherd (1985).

Unreduced prolamins were extracted by treating crushed endosperm halves in about 0.8 ml of 70% aqueous ethanol (pH 5.9) at 50 °C for about 5 h. The top portion of the supernatant was collected after 5 min of centrifugation at 10,000 rpm and was dried at 60 °C by allowing the ethanol to evaporate. The residue (dried pellet) was re-dissolved in 80 µl of TRIS-HCl buffer containing 4% SDS, and 40–70 µl of the extract was loaded into each slot of the gel for two-step electrophoresis.

*Electrophoresis*

SDS-polyacrylamide gel electrophoresis used was as described before (Gupta et al. 1989), except that the two-step SDS-PAGE was also performed on hot ethanol extracts. In the first step of the two-step procedure, unreduced prolamins (gliadins and glutenins) were run for about 1 h in a 10% acrylamide gel, and then the top 1-cm portion across the separating gel (containing glutenins) was cut and equilibrated for about 2 h at 50 °–60 °C in an equilibration solution (pH 6.8) containing 2% 2-mercaptoethanol, to reduce the disulphide linkages. In the second step, this gel strip (now containing reduced subunits of glutenin) was loaded onto another gradient gel (7.5%–15.0% acrylamide) and was electrophoresed at a constant current of 25 mA/gel for 6 h.

## Results

*Characterization of endosperm proteins by two-step 1-D SDS-PAGE*

When proteins extracted in TRIS-HCl buffer (pH 6.8) containing SDS were separated by two-step SDS-PAGE, they were resolved into three groups of bands with different electrophoretic mobility (slow, medium, and fast) in the second step of separation (Fig. 1). The slowest moving bands correspond to the A subunits of glutenin, and the majority of the fast-moving bands corresponds to B and C subunits of glutenin. However, when these patterns were compared with those obtained with hot ethanol extracts (Fig. 2), it became evident that all the bands with medium mobility and some of the fastest moving bands are non-prolamins, and these have been arbitrarily divided into HMW and LMW components, respectively (Fig. 1). Some of these non-prolamin bands have similar mobilities to the B and C subunits and they obscure the resolution of these bands, particularly the C subunits, and thus only the B subunit variation was described in the previous report (Gupta and Shepherd 1988). The absence of these non-prolamin bands in the ethanol extracts has greatly improved the resolution of the LMW glutenin subunits into two clear groups representing B and C subunits (Fig. 2). We have now used the ethanol extraction procedure to re-analyze the 207 cultivars previously extracted with SDS solution and have included another 15 cultivars. The B subunits were well resolved with this procedure, but the C subunits were usually faintly stained and their variation could be analyzed in only 125 of the 222 cultivars studied.

*Intervarietal variation in B and C subunits of glutenin and its genetic control*

The B and C subunit pattern was determined on at least six seeds of each cultivar and several reference cultivars,

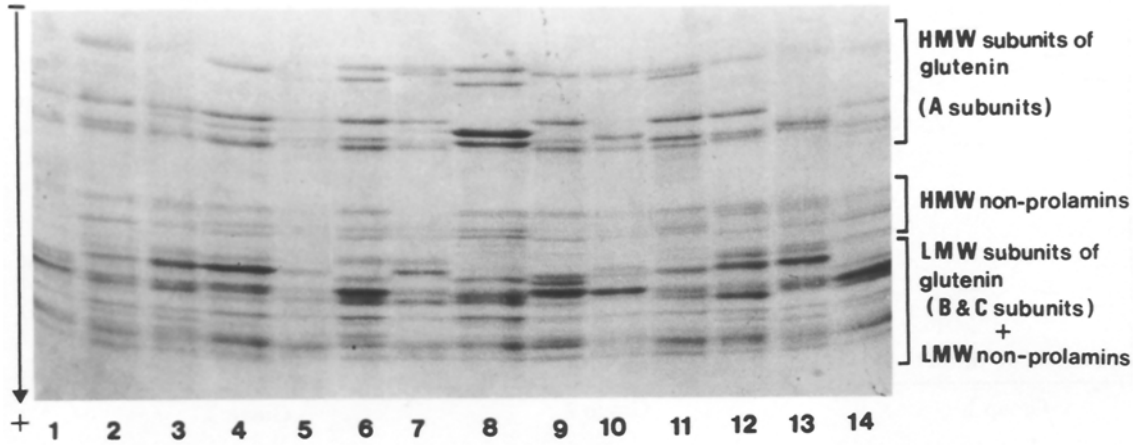


**Fig. 1.** Two-step 1-D SDS-PAGE patterns of endosperm proteins extracted in TRIS-HCl buffer (pH 6.8) containing SDS from hexaploid wheat cultivars: (*1*) Chinese Spring, (*2*) Tincurrin, (*3*) Chhoti Lerma, (*4*) Giza-150, (*5*) Lovrin-13, (*6*) Nanbukomugi, (*7*) Orca, (*8*) Insignia, (*9*) Chinese Spring, (*10*) Gabo, (*11*) Kozara, (*12*) Norin-61, (*13*) Tanori-71, and (*14*) Vicam-71

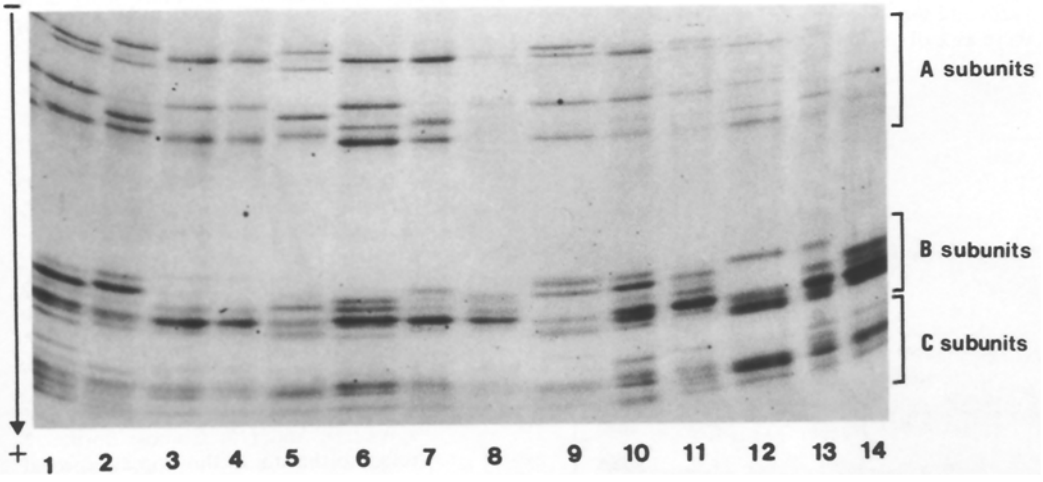


**Fig. 2.** Two-step 1-D SDS-PAGE patterns of endosperm proteins extracted in hot 70% aqueous ethanol from hexaploid wheat cultivars: (*1*) Arzu, (*2*) BT-2296, (*3*) Bencubbin, (*4*) Bungulla, (*5*) Halberd, (*6*) Chinese Spring, (*7*) Gabo, (*8*) Cheyenne, (*9*) Orca, (*10*) Tincurrin, (*11*) Timgalen, (*12*) Cebecco-97, (*13*) Chanab-70, and (*14*) Una. Note that HMW and LMW non-prolamin bands are not extracted in this solvent

viz., Chinese Spring, Gabo, Halberd, Cheyenne, Norin-61, and Orca, were included to determine the relative mobilities of these subunits. Extensive variation in subunit pattern was observed (Fig. 2) and a total of 40 clearly scorable bands with different mobility (28 B and 12 C subunits) could be identified. Each cultivar, with the exception of those carrying a *1BL-1RS* translocation, possessed from 7 to 16 different B and C subunits.

Examination of the banding patterns of these cultivars revealed that some LMW bands, or band combinations, were not present together in the same cultivar, i.e., the patterns occurred as alternatives to each other. Based on their mutual exclusiveness and the available inheritance data (Gupta and Shepherd 1988; Gupta 1989), these subunit bands were assigned to three groups (Fig. 3). The chromosomal control of these patterns was determined by analyzing the intervarietal and intergeneric substitution lines, and the results obtained clearly indicated that patterns within each group were controlled by genes on the same chromosome. Six patterns were assigned to group 1 (Fig. 3). Pattern 'a' and 'b' correspond to Chinese Spring (CS) and Gabo bands, respectively, and are controlled by genes on chromosome arm *1AS* (Gupta and Shepherd 1987, 1988). Additional evidence that pattern 'b' is controlled by chromosome *1A* came from the analysis of the CS-Kenya Farmer *1A* substitution line (Fig. 4, slot 2), which possesses chromosome *1A* from Kenya Farmer. This line possessed pattern 'b' (see bands marked by ►) instead of pattern 'a' of Chinese Spring. Similarly, in CS-Cheyenne *1A* (Fig. 5,

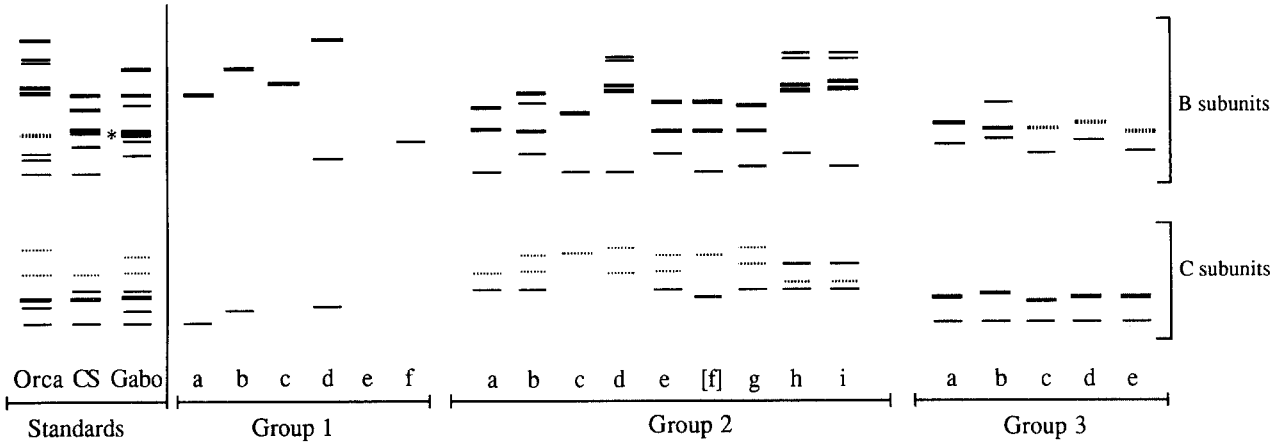


**Fig. 3.** Diagram showing the three groups of B and C subunit or subunit combinations identified by two-step SDS-PAGE analysis of 222 bread wheat cultivars. The grouping is based on the mutual exclusiveness of these bands or band combinations among the cultivars. This diagram also incorporates the information obtained on these subunits from analyses of substitution lines and the test-cross progeny. Patterns 'a' and 'b' in each group are from Chinese Spring and Gabo, respectively. [f] = direct evidence for the chromosomal location of pattern 'f' has not been obtained. The thickness of bars in the patterns indicates the approximate thickness of the bands. Faintly stained bands are shown by *broken lines*. * denotes that this thick band represents two bands of the same mobility, one controlled by *1BS* and the other by *1DS* in Chinese Spring and Gabo. These two bands have been included in group 2 and group 3 patterns of these as well as other cultivars having the denoted thick band

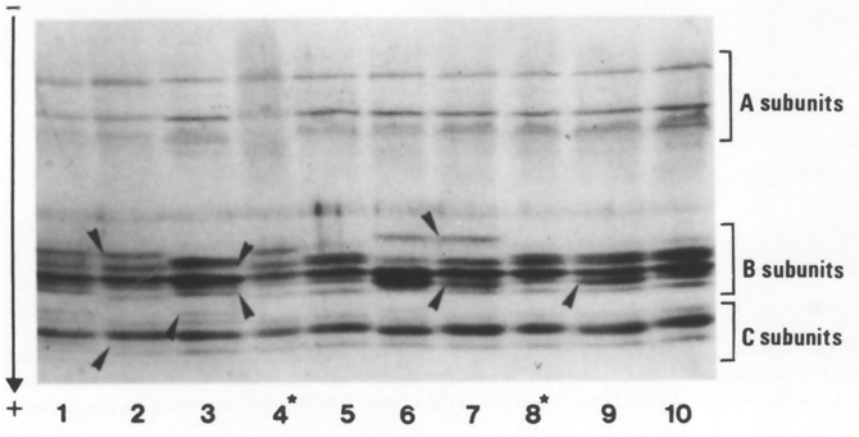


**Fig. 4.** Two-step 1-D SDS-PAGE patterns of protein extracted in hot 70% aqueous ethanol from bread wheat cultivars and intervarietal substitution lines: (*1*) Kenya Farmer, (*2*) Chinese Spring (CS)-Kenya Farmer *1A*, (*3*) CS-Kenya Farmer *1B*, (*4**) CS-Kenya Farmer *1D*, (*5*) Chinese Spring, (*6*) Capelle Desprez, (*7*) CS-Capelle Desprez *1A*, (*8**) CS-Capelle Desprez *1B*, (*9*) CS-Capelle Desprez *1D*, and (*10*) Chinese Spring. * refers to the stocks showing unexpected protein phenotypes. ► indicates LMW subunits substituted for those of Chinese Spring controlled by chromosomes *1A*, *1B*, and *1D*

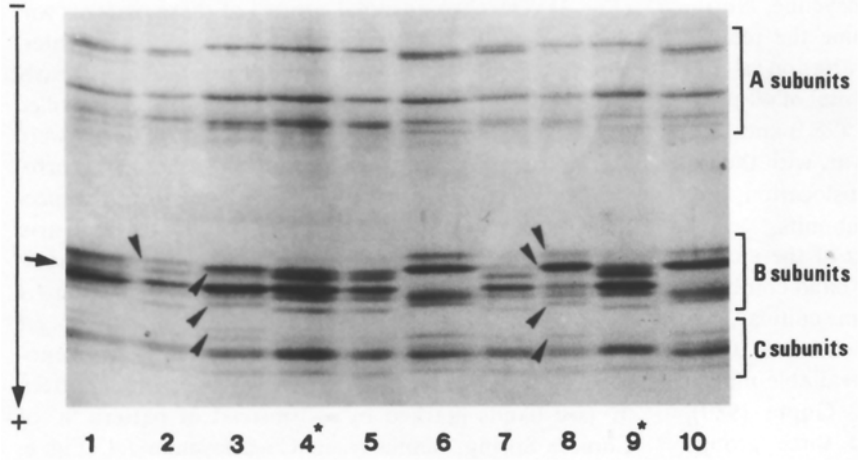


**Fig. 5.** Two-step 1-D SDS-PAGE patterns of protein extracted in hot 70% aqueous ethanol from bread wheat cultivars and intervarietal substitution lines: (*1*) Cheyenne, (*2*) Chinese Spring (CS)-Cheyenne *1A*, (*3*) CS-Cheyenne *1B*, (*4**) CS-Cheyenne *1D*, (*5*) Chinese Spring, (*6*) Thatcher, (*7*) CS-Thatcher *1A*, (*8*) CS-Thatcher *1B*, (*9**) CS-Thatcher *1D*, and (*10*) Thatcher. → refers to a unique B subunit in Cheyenne possibly controlled by *1D*

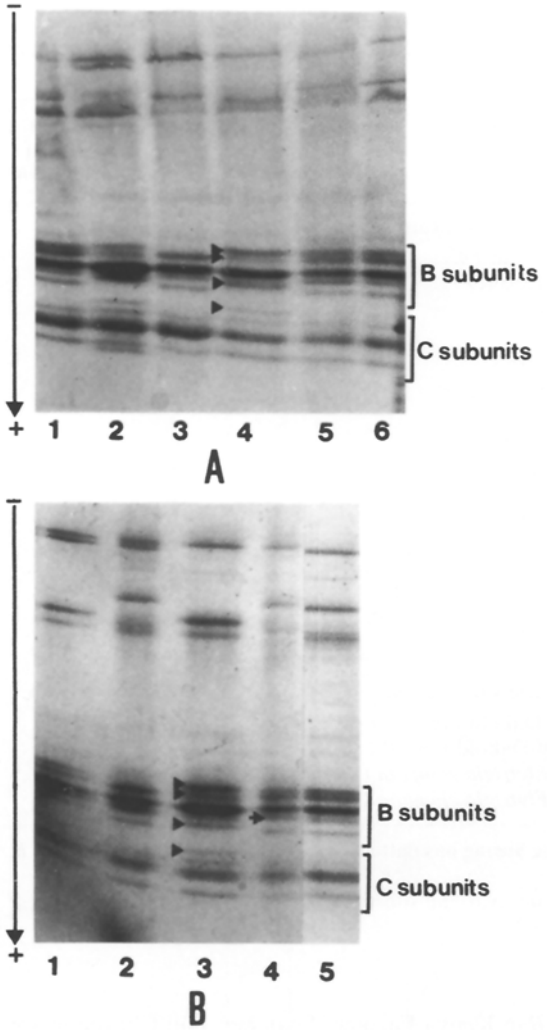


**Fig. 6 A and B.** Two-step 1-D SDS-PAGE patterns of protein extracted in 70% aqueous ethanol from bread wheat cultivars and intervarietal substitution lines. **A** (*1, 6*) Chinese Spring (CS), (*2*) Hope, (*3*) CS-Hope *1A*, (*4*) CS-Hope *1B*, and (*5*) CS-Hope *1D*. **B** (*1*) Timstein, (*2*) CS-Timstein *1A*, (*3*) CS-Timstein *1B*, (*4*) CS-Timstein *1D*, and (*5*) Chinese Spring. ▸ refers to LMW subunits in pattern 'b' (group 2) controlled by chromosome *1B* in Hope and Timstein. → indicates LMW subunit of pattern 'b' (group 3) controlled by chromosome *1D* in Timstein

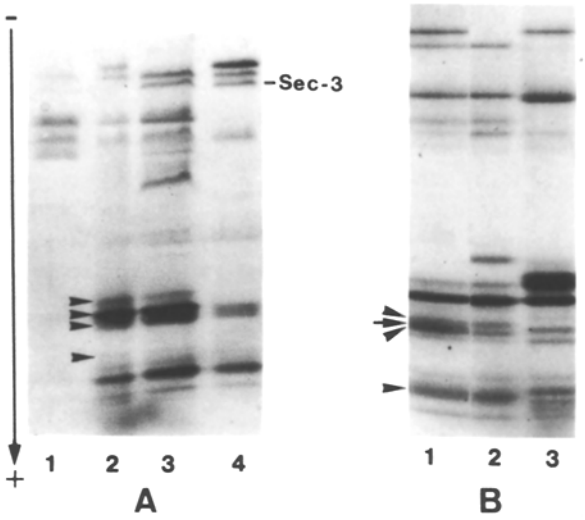


**Fig. 7 A and B.** Two-step 1-D SDS-PAGE patterns of proteins extracted in hot 70% aqueous ethanol. **A** (*1*) Dakold rye, (*2*) Kharkov wheat, (*3*) Kharkov-Dakold rye *3R* (*1A*) and (*4*) Kharkov-Dakold rye *3R* (*1B*). **B** (*1*) Rescue, (*2*) Rescue-*E. elongata* substitution line (*1A*), and (*3*) Rescue-*E. elongata* substitution line (*1D*). ► and → indicate LMW subunits controlled by group 1 chromosomes in Kharkov and Rescue

slot 2) and CS-Capelle Desprez *1A* (Fig. 4, slot 7), substitution lines revealed that pattern 'c' from Cheyenne and pattern 'd' from Capelle Desprez replaced 'a' in Chinese Spring (see bands marked by ► in the respective slots). Inheritance tests have shown that pattern 'd' in cultivar Orca is controlled by genes on chromosome arm *1AS* (Gupta 1989). Many cultivars did not have any of the bands known to be controlled by chromosome *1A*, and thus they were classified as null phenotypes (pattern 'e'). For example, the substitution lines carrying chromosome *1A* from cultivars Hope (Fig. 6A, slot 3), Thatcher (Fig. 5, slot 7), and Timstein (Fig. 6 B, slot 2) in a Chinese Spring background lacked pattern 'a' and did not show any extra band. It should be noted, however, that the faster moving band in pattern 'a' usually overlaps with a band controlled by *1DS* in Chinese Spring (Gupta 1989), and thus it is not completely absent in these substitution lines. Kharkov also carries the null phenotype, since the reported substitution line Kharkov-Dakold rye *3R* (*1A*) (Fig. 7 A, slot 3) had the same band composition as the Kharkov parent (Fig. 7 A, slot 2). A similar analysis of a Rescue-*Elytrigia elongata* (*1A*) substitution line (Fig. 7 B, slot 2) revealed that pattern 'f' in Rescue (band shown by →) was controlled by genes on chromosome *1A*.

Nine different patterns have been assigned to group 2 and each pattern had two or more B subunit bands (Fig. 3). Patterns 'a' and 'b' were controlled by chromosome arms *1BS* in Chinese Spring and Gabo, respectively (Gupta and Shepherd 1987, 1988). The banding patterns of Chinese Spring substitution lines having chromosome *1B* from Hope (Fig. 6 A, slot 4), Kenya Farmer (Fig. 4, slot 3), or Timstein (Fig. 6 B, slot 3) also showed that pattern 'b' in these cultivars is controlled by genes on chromosome *1B* (see bands shown by ► in the respective slots). Patterns 'c' and 'd' were present in cultivars Insignia and Orca, respectively, and were also controlled by genes on chromosome arm *1BS* (Gupta and Shepherd 1988; Gupta 1989). Cultivar Cheyenne possesses pattern 'e', which replaced the pattern 'a' of Chinese Spring in the CS-Cheyenne *1B* substitution line (bands shown by ►, Fig. 5, slot 3) thus indicating that pattern 'e' was controlled by chromosome *1B*. Similar analysis of Kharkov-Dakold rye *3R* (*1B*) (Fig. 7 A, slot 4) and CS-Thatcher *1B* (Fig. 5, slot 8) substitution lines showed that genes

controlling pattern 'g' in Kharkov (bands shown by ►) and pattern 'h' in Thatcher (bands shown by ►) were located on chromosome *1B*. The inheritance test revealed that genes controlling pattern 'i' in Norin-61 were located on *1BS* (Gupta 1989). Direct evidence for control of pattern 'f' by this chromosome has not yet been obtained. Ten of the cultivars analyzed did not exhibit any of these group 2 patterns, and the presence of rye secalins controlled by chromosome *1R* (data not shown) suggested that they carried a *1B/1R* substitution (viz., Neuzucht, Saladin) or *1BL-1RS* translocations (viz., Cebecco-97, Kozara, Licanka, Lovrin-13, Macvanka-2, Posavka-1, Wembley, Zelengora).

The five different patterns allocated to group 3 (Fig. 3) were associated with chromosome *1D*. Patterns 'a' and 'b' corresponding to the bands in Chinese Spring and Gabo, respectively, were controlled by genes on the short arms of chromosome *1D* (Gupta and Shepherd 1987, 1988). Pattern 'a' was also present in cultivar Rescue and was found to be absent in Rescue-*E. elongata* (*1D*) substitution line (bands marked by ►, Fig. 7 B, slot 3), thus indicating its genetic control by chromosome *1D* in this cultivar as well. Timstein possessed pattern 'b', which replaced pattern 'a' in CS-Timstein *1D* substitution line (Fig. 6 B, slot 4), and thus confirmed its genetic control by chromosome *1D*. Similarly, by analyzing the CS-Capelle Desprez *1D* substitution line (Fig. 4, slot 9) and by inheritance analysis of Insignia (Gupta and Shepherd 1988), the genetic control of pattern 'c' in cultivar Capelle Desprez (band indicated by ►) and Insignia was determined. Patterns 'd' and 'e' were both found in cultivar Norin-61 and the inheritance test revealed that they were controlled by genes on chromosome *1D* (Gupta 1989). The data obtained on the chromosomal control of the different patterns in these cultivars from analysis of substitution lines are summarized in Table 1.

The HMW glutenin subunit and gliadin (figure not shown) patterns of these genetic stocks were also analyzed to confirm their authenticity, and the results were generally as expected. An exception occurred where seeds designated CS-Kenya Farmer *1D* substitution (Fig. 4, slot 4) had the same protein phenotype as the CS-Kenya Farmer *1A* substitution. Similarly, substitution lines CS-Thatcher *1D* (Fig. 5, slot 9), CS-Capelle Desprez *1B* (Fig. 4, slot 8), and CS-Cheyenne *1D* (Fig. 5, slot 4) gave phenotypes similar to Chinese Spring, and it appears that these stocks may have been incorrectly labelled. Although the CS-Cheyenne *1A* line (Fig. 5, slot 2) showed the expected differences for chromosome *1A* substitution, it also possessed Cheyenne *1D*-HMW subunits, probably retained through insufficient backcrossing. Even though the authentic lines were not available, it was possible to deduce from the donor parent pattern which bands are controlled by the chromosome involved. Thus, it was deduced that the bands that were not controlled by *1A* or *1B* in Kenya Farmer, Thatcher, and Cheyenne are controlled by *1D*, and they formed patterns 'a', 'e' (Fig. 3), and 'f' (not shown in Fig. 3), respectively. Pattern 'f' of Cheyenne is considered to be an additional pattern to those already classified in group 3 because one of the bands possessed an unusual mobility, as indicated by → (Fig. 5). The bands not controlled by *1A* and *1D* in Capelle Desprez (pattern 'g', group 2) were assigned to chromosome *1B*.

It has been suggested that the supposed substitution of *3R* of Dakold rye for chromosomes *1A* and *1B* of Kharkov wheat might instead be a *1R/3R* translocation (Gupta 1969; Lee et al. 1969) and the data presented here support this notion. Thus, the lines designated *3R* (*1A*) and *3R* (*1B*) substitution show the expected loss of chromosomes *1A*- and *1B*-controlled HMW and LMW subunits of glutenin (Fig. 7 A) and gliadins (Gupta 1989) from Kharkov, but possess the HMW secalin band Sec-3 (Fig. 7 B) known to be controlled by chromosome arm *1RL* in Imperial rye (Lawrence and Shepherd 1981). They do not exhibit the Sec-1 bands controlled by chromosome arm *1RS*. That is, these lines must be a *1RL/3R* translocation.

**Table 1.** Chromosomal control of LMW subunits of glutenin as inferred from the analysis of intervarietal and intergeneric substitution lines

| Genetic stocks | LMW glutenin subunit pattern added or deleted[a] |
|---|---|
| Intervarietal substitutions[b] | Pattern added |
| Chromosome *1A* | |
| Capelle-Desprez | d |
| Cheyenne | c |
| Hope | e |
| Kenya Farmer | b |
| Thatcher | e |
| Timstein | e |
| Chromosome *1B* | |
| Cheyenne | e |
| Hope | b |
| Kenya Farmer | b |
| Thatcher | h |
| Timstein | b |
| Chromosome *1D* | |
| Capelle Desprez | c |
| Timstein | b |
| Intergeneric substitutions | Pattern deleted |
| Karhov Dakold rye *3R* (*1A*) | None |
| Kharkov-Dakold rye *3R* (*1B*) | g |
| Rescue-*Elytrigia elongata* (*1A*) | f |
| Rescue-*Elytrigia elongata* (*1D*) | a |

[a] Chinese Spring has pattern 'a' for all the chromosomes *1A*, *1B*, and *1D*

[b] These intervarietal substitution lines are present in a Chinese Spring background

**Table 2.** Hexaploid wheat cultivars heterogeneous for LMW subunit (B and C subunits) composition

| Cultivar | Biotype | Seeds tested | LMW subunit pattern | | |
|---|---|---|---|---|---|
| | | | *1A* | *1B* | *1D* |
| Bayonet | A | 4 | c | b | b |
| | B | 4 | e | b | b |
| BT-2288 | A | 9 | f | i | a |
| | B | 8 | e | i | a |
| C-306 | A | 3 | c | b | a |
| | B | 6 | c | i | a |
| Condor | A1 | 19 | c | b | b |
| | A2 | 27 | b | b | b |
| Gamenya | A | 13 | b | b | b |
| | B | 16 | c | b | b |
| Huelquen | A | 5 | c | g | c |
| | B | 4 | b | g | c |
| Norin-61 | A | 14 | e | i | d |
| | B | 17 | d | i | e |
| Opal | A | 6 | e | g | a |
| | B | 8 | e | b | a |
| Potam-70 | A | 6 | c | g | a |
| | B | 11 | c | i | a |
| Okukomugi | A | 3 | d | d | e |
| | B | 4 | e | d | e |
| Timstein | A | 2 | c | b | b |
| | B | 4 | e | b | b |
| Toquifen *S* | A | 3 | e | b | c |
| | B | 3 | e | h | b |
| 8156 WG | A | 9 | c | g | b |
| | B | 2 | b | g | b |

*Intravarietal variation in LMW subunit composition*

Thirteen of the 222 cultivars were found to consist of two biotypes with respect to B and C subunits (Table 2). Biotypes of the cultivars Bayonet, BT-2288, Condor, Gamenya, and Norin-61 were grown and found to have similar or identical plant morphology, so the different patterns within these cultivars represented true biotypes and were not the result of seed mixing or chance outcrossing. The other biotypes were not compared for plant morphology, but since most exhibited very similar banding patterns for all of the other seed proteins analyzed (not shown), it was assumed that they were biotypes. A low proportion of seeds of Potam-70 and Huelquen, however, showed other banding patterns for LMW subunits of glutenin besides those in Table 2, and since these seeds also possessed different HMW subunits, gliadins, and triplet bands (not shown), they were considered to be seed contaminants.

## Discussion

Although glutenin in its native state has been defined as being insoluble in aqueous ethanol (Osborne 1907), the fraction known as LMW glutenin is readily soluble in 70% ethanol at room temperature or even lower (4 °C) temperatures (Nielsen et al. 1968; Bietz and Wall 1973; Byers et al. 1983), and this fraction consists of B and C subunits (Payne and Corfield 1979). In contrast, native HMW glutenins (consisting of A, B and C subunits) are either insoluble (Byers et al. 1983) or sparingly soluble (Bietz and Wall 1973) in ethanol at room temperatures. The two-step electrophoretic patterns of proteins extracted in 70% aqueous ethanol at higher temperatures ($\geq 50$ °C) revealed that glutenins containing A, B, and C subunits were readily soluble in their native state. The quantitative differences in the solubility of native HMW glutenins at these temperatures can be attributed to the molecular weights of the glutenins (Graveland et al. 1985). Since both covalent (inter-chain disulphide bonds) and noncovalent forces (hydrophobic bonds etc.) are involved in the stabilization of glutenin polymers (Khan and Bushuk 1979), it is likely that at higher temperatures, these forces between the polymers may be disrupted (Schofield et al. 1983), thus causing reduction in the sizes of HMW glutenins and hence the increased solubility. The data presented here clearly indicate that HMW glutenins, like gliadins, are soluble in ethanol in their native state, and hence can be included in the prolamin class as proposed by Shewry et al. (1986).

Fractionation of the ethanol-soluble glutenins by the two-step method provided much improved resolution of LMW glutenin subunits because of the exclusion of nonprolamins which overlapped these subunits. This allowed a total of 20 LMW subunit band patterns to be detected in the 222 cultivars analyzed. Genetic evidence presented here and elsewhere (Gupta and Shepherd 1987, 1988; Gupta 1989) has indicated that 6, 9, and 5 of these patterns were controlled by allelic genes at *Glu-A3*, *Glu-B3*, and *Glu-D3* loci on the chromosome arms *1AS*, *1BS*, and *1DS*, respectively. The cultivars listed in Table 3 are recommended for use as standards for each pattern/allele.

The band patterns of all 222 bread wheat cultivars analyzed are described elsewhere (Gupta 1989). In the analysis of the LMW subunit patterns of these cultivars, however, some difficulties were encountered. The Glu-B3 bands (group 2, Fig. 3) represented a wide range of mobilities, and some of them overlapped with Glu-A3 and Glu-D3 bands (group 1 and 3, Fig. 3). For example, the four slowest moving Glu-B3 bands in patterns 'd', 'h', and 'i' often separated as two distinctly different thick bands, and the faster one has similar mobility to a Glu-A3 band in patterns 'a' and 'c'. Similarly, the slowest Glu-B3 band in pattern 'b' (group 2) often coincided with that of pattern Glu-A3a (group 1), but the former was usually paler and thinner, which helped to distinguish between them. Many cultivars had the thickest B subunit band (shown by * in Fig. 3) but this did not show mutual exclusiveness with any other bands. Further studies have

**Table 3.** Cultivars recommended as standards for each pattern controlled by *Glu-A3*, *Glu-B3*, and *Glu-D3* loci

| Locus | Pattern | Standards |
|---|---|---|
| *Glu-A3* | a | Chinese Spring |
| | b | Gabo |
| | c | Cheyenne |
| | d | Capelle Desprez, Orca |
| | e | Hope, Insignia |
| | f | Rescue |
| *Glu-B3* | a | Chinese Spring |
| | b | Gabo, Timstein, Hope |
| | c | Insignia, Halberd |
| | d | Orca |
| | e | Cheyenne |
| | f | Radja |
| | g | Kharkov, Bungulla |
| | h | Thatcher, Rescue |
| | i | Norin-61 |
| *Glu-D3* | a | Chinese Spring |
| | b | Gabo |
| | c | Insignia, Capelle Desprez |
| | d | Norin-61A |
| | e | Orca, Thatcher |

revealed that this band represented two subunits in cultivars Gabo and Chinese Spring, one controlled by *1BS* and the other by *1DS* (Gupta and Shepherd 1987) and, hence, this could not be assigned to any group. Based on this information, it was assumed for simplicity that this band in other cultivars was also controlled by *1BS*+*1DS* (although this may not be true for all the cultivars) and, hence, one band of similar mobility to this has been included in the respective group 2 (Glu-B3) and 3 (Glu-D3) patterns of all the cultivars exhibiting it. This composite band sometimes joined the adjacent faster moving B subunit in pattern Glu-D3b and formed an even thicker band. This composite band was, however, missing from most of the cultivars having Glu-D3 patterns 'c', 'd', and 'e' and in some cases only a faint band was present in the background.

The number of band combinations found in the LMW glutenin subunits of hexaploid wheats is much lower than the expected number of such combinations on the basis of random association, indicating that genes coding for these bands are closely linked. Such close linkage has been demonstrated by Singh and Shepherd (1988) by detecting a low level of recombination between LMW glutenin subunits on chromosome *1B*. The exact genetic and molecular nature of variation of LMW glutenin subunits is not known yet; however, rare recombination and point mutation can generate new LMW subunit combinations. Moreover, the analysis of nucleotide sequences of a LMW glutenin gene has revealed the presence of repetitive sequences (Colot et al. 1989), and if differences in their sizes and numbers exist between the genes, unequal crossing-over between these repeats might produce new subunits and will thus contribute towards the multiplicity and variation of LMW glutenin subunits.

The extent of variation in LMW subunit patterns for each chromosome *1A*, *1B*, and *1D* described here is similar to that reported for these chromosomes with respect to gliadins and HMW subunits of glutenin (Sozinov and Poperelya 1980; Payne and Lawrence 1983; Galili and Feldman 1983). All these proteins have shown greatest polymorphism for chromosome *1B* and are consistent with the hypothesis that the B genome in polyploid wheat is polyphyletic (Sarkar and Stebbins 1956; Athwal and Kimber 1972). The alternative hypothesis of a monophyletic origin of the B genome in polyploid wheats (Riley 1965), however, cannot be rejected since the B genome may have undergone preferential changes during the evolution of the polyploid wheats. As a result, genes on chromosome *1B* coding for these storage proteins might have duplicated and diverged more extensively than the genes on chromosomes *1A* and *1D*.

The results also showed that chromosome *1A* encoded for a minimum number of LMW subunits and many cultivars did not exhibit any band controlled by this chromosome. Interestingly, this chromosome also controls a minimum number of HMW subunits of glutenin and the gliadins in bread wheat, and it has been suggested that massive nonrandom diploidization and silencing of genes have occurred on chromosome *1A* (Galili and Feldman 1983). Recent data have indicated that the gene for HMW glutenin null phenotype is transcriptionally inactive due to a single base-pair substitution within 280 bp of sequence immediately upstream of the transcription start site (Halford et al. 1989). However, it is not known why such change has occurred so preferentially on chromosome *1A*.

In addition to providing information about the evolution of wheat, the genes controlling seed proteins in wheat are of practical value. They serve as useful protein markers for the identification of cultivars/varietal mixtures (Wrigley et al. 1982), as genetic markers for the identification of chromosomes and chromosome arms, which can greatly facilitate the transfer of alien genetic material into bread wheat (Lawrence and Shepherd 1981; Koebner and Shepherd 1988). The LMW glutenin subunits will thus serve as additional protein (genetic) markers for these purposes. More importantly, allelic differences in the LMW glutenin subunits have recently been shown to be significantly related to flour qualities in bread (Gupta and Shepherd 1987, 1988; Gupta et al. 1989; Payne et al. 1987) and durum wheats (Autran et al. 1987). However, much more information is required on the relationships between the different LMW glutenin subunit alleles and technological qualities of wheat flours. Eventually, it may be possible to select for im-

proved quality on the basis of LMW glutenin subunit composition. Similarly, re-introduction of the chromosome *1B*-controlled LMW glutenin subunits that are lost in *1B/1R* substitution and translocation lines might alleviate the poor dough quality of these lines (Dhaliwal et al. 1988; Koebner and Shepherd 1988).

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