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

2. Genetic control of the subunits in species related to wheat

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Summary. Analysis of intergeneric substitution lines in hexaploid wheats by a two-step electrophoretic method of protein separation revealed that low-molecular-weight (LMW) subunits of glutelin in *Triticum longissimum*, *T. umbelullatum*, *Elytrigia elongata* (2×) were controlled by chromosomes/chromosome arms *1S*^{l}, *1U*, and *1ES*, respectively. A LMW glutelin band in *Secale montanum* was detected but its chromosomal location could not be determined. Genes controlling gliadins and HMW subunits of glutelin were also located on chromosome *1S*^{l} in *T. longissimum*.

Key words: *Triticum longissimum* – *T. umbelullatum* – *Elytrigia elongata* – LMW subunits of glutelin – Chromosomal location

Introduction

It has been shown that the LMW subunits of glutenin are controlled by genes on the short arms of group 1 chromosomes in wheat (Jackson et al. 1983; Gupta and Shepherd 1987; Singh and Shepherd 1988), and that they exhibit extensive variation in banding pattern at each ploidy level of wheat (Gupta and Shepherd 1988; Gupta 1989). However, the pattern variation and the chromosomal location of the genes controlling these subunits in species related to wheat have not yet been determined. We have now used intergeneric substitution lines to determine the genetic control of these subunits in *T. umbellulatum*, *T. longissimum*, and *Elytrigia elongata* (2×), and the results are reported herein. Some *Secale* species and the addition lines involving individual chromosomes from *Secale montanum* were also analyzed.

Materials and methods

Genotypes analyzed

The species nomenclature used is that of Morris and Sears (1967) except *Agropyron elongatum* has been described as *Elytrigia elongata* following Dvorak (1981).

One accession each of *T. umbellulatum* (2n=14, UU), *T. longissimum* (2n=14, $S^{l}S^{l}$), and *Elytrigia elongata* (2n=14, EE) and the following genetic stocks involving chromosomes from these species in bread wheat were examined: CS × *T. umbellulatum* amphiploid (Kimber 1967) and substitution lines 1*U* (1*A*), 1*U* (1*B*), and 1*U* (1*D*) (Shepherd 1973); CS × *T. longissimum* substitution $1S^{l}$ (1*B*) (Netzle and Zeller 1984); CS × *Elytrigia elongata* amphiploid stock and substitution lines 1*E* (1*A*), 1*E* (1*B*), 1*ES* (1*B*), 1*E* (1*D*), and 1*ES* (1*D*) (Dvorak and Sosulski 1974; Dvorak 1980).

Seeds from five cultivars of rye (*Secale cereale*), namely, Imperial, King-II, South Australian, Petkus, and Dakold and two accessions (R-15, R-42) of wild rye (*Secale montanum*) plus the genetic stocks CS × *S. montanum* R-15 amphiploid and four addition lines having chromosomes $1R^{m}$, $1R^{m}S$, $2R^{m}$, and $6R^{m}$ added to Chinese Spring wheat (Miller, T. unpublished data) were analyzed.

Protein extraction and electrophoresis

The procedures used for extraction and electrophoresis of endosperm proteins and staining and destaining the gels were as described earlier (Gupta et al. 1989a; Gupta and Shepherd 1990).

Results

T. longissimum

The chromosomal control of LMW glutelin bands in *T. longissimum* was determined by comparing the band-

* The term 'glutelin' refers to the polymeric prolamins of cereals, e.g., glutenins in wheat, HMW, and 75-k secalins in rye

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ing patterns of the Chinese Spring-*T. longissimum* $1S^l$ (*1B*) substitution line and Chinese Spring (Fig. 1 A). The seeds of *T. longissimum* and the Chinese Spring-*T. longissimum* amphiploid used to produce this substitution line were not available for analysis. However, another accession of *T. longissimum* was available and it showed at least seven LMW subunits of glutelin (Fig. 1 A, slot 1). The substitution line (Fig. 1 A, slot 3) possessed three extra LMW glutelin bands (S4, S5, S6), while the chromosome *1B*-controlled LMW subunits of Chinese Spring (Gupta and Shepherd 1987) were absent, as expected. One of the B subunit bands in this stock had the same mobility as a *1B*-controlled LMW subunit of Chinese Spring, but this was probably derived from *T. longissimum* also (band S3). If so, four LMW subunits of glutelin are controlled by chromosome $1S^l$ in *T. longissimum*. This stock also had two extra HMW subunits of glutelin (S1 and S2, Fig. 1 A, slot 3) and four extra gliadin bands (S7, S8, S9, S10, Fig. 1 B, slot 3). The arm location of the genes controlling these protein bands has not yet been determined.

T. umbellulatum

The two-step banding patterns of an accession of *T. umbellulatum*, Chinese Spring, and the amphiploid between *T. umbellulatum* are given in Fig. 2. The banding pattern of *T. umbellulatum* exhibited nine subunits of glutelin. Although this particular accession is not the one used to produce the amphiploid (K. W. Shepherd, personal communication), it has four bands with the same mobilities as bands U1, U2, U3, and U5 in the amphiploid (Fig. 2, slot 3). An additional band (U4) was present in the amphiploid. Bands U4 and U5 correspond to LMW subunits of glutelin, while the others are HMW subunits. Analysis of the banding patterns of the substitution lines *1U* (*1D*) (Fig. 2, slot 4), *1U* (*1B*) (Fig. 2, slot 5), and *1U* (*1A*) (Fig. 2, slot 6) showed that these bands were added, while the LMW and HMW glutelin bands controlled by chromosomes *1A*, *1B*, and *1D* in Chinese Spring (Gupta and Shepherd 1987) were deleted, respectively, in these stocks. Thus, chromosome *1U* controls two LMW subunits (U4, U5) and three HMW subunits (U1, U2, U3) of glutelin, as shown in Fig. 2. The chromosomal control of HMW subunits of glutelin is in agreement with the previous finding of Lawrence and Shepherd (1981), and previously it had been shown that chromosome *1U* carries genes for gliadins (Shepherd 1973).

Elytrigia elongata (2×)

The two-step band patterns of the amphiploid involving *E. elongata* (2n = 14) and Chinese Spring wheat and various substitution lines involving replacement of group 1

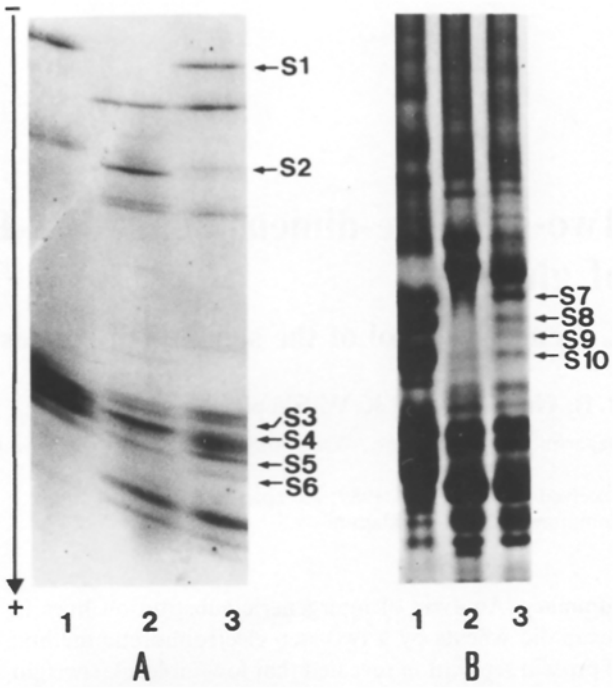

Fig. 1 A and B. Fractionation by two-step SDS-PAGE (**A**) and one-dimensional SDS-PAGE (**B**) of 70% ethanol-extracted endosperm proteins from (*1*) *Triticum longissimum* (2n = 14, S^1 S^l). (*2*) Chinese Spring, and (*3*) Chinese Spring-*T. longissimum* $1S^l$ (*1B*) substitution line

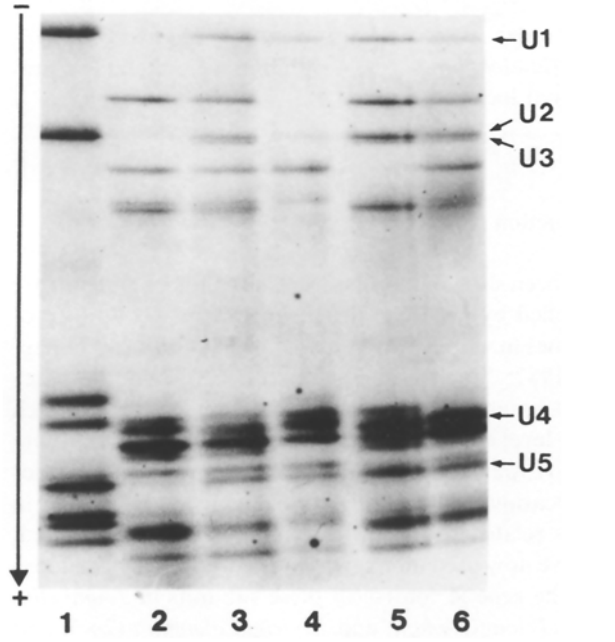

Fig. 2. Two-step SDS-PAGE patterns of 70% ethanol-extracted seed proteins from (*1*) *Triticum umbellulatum* (2n = 14, UU), (*2*) Chinese Spring, (*3*) Chinese Spring-*T. umbellulatum* amphiploid (2n = 56), and Chinese Spring-*T. umbellulatum* substitution lines (*4*) *1U* (*1D*), (*5*) *1U* (*1B*), and (*6*) *1U* (*1A*)

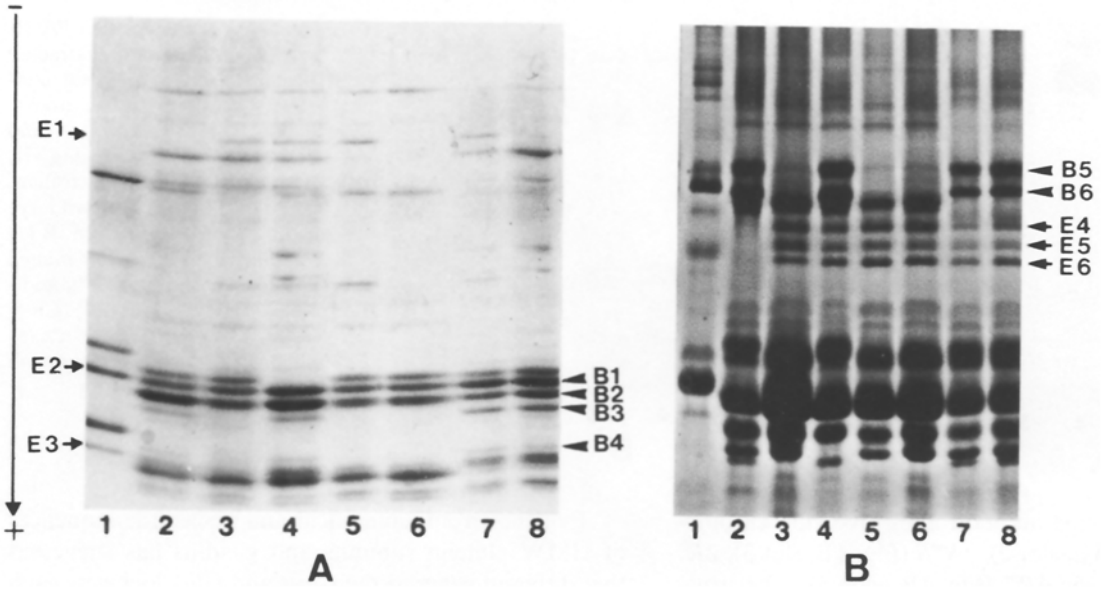

Fig. 3 A and B. Fractionation by two-step SDS-PAGE (**A**) and 1-D SDS-PAGE (**B**) of 70% ethanol-extracted seed proteins from (*1*) *Elytrigia elongata* (2n=14, EE), (*2*) Chinese Spring, (*3*) Chinese Spring-*E. elongata* amphiploid, and Chinese Spring-*E. elongata* substitution lines (*4*) *1E* (*1A*), (*5*) *1E* (*1B*), (*6*) *1ES* (*1B*), (*7*) *1E* (*1D*) and (*8*) *1ES* (*1D*)

wheat chromosomes are shown in Fig. 3 A. The accession of *E. elongata* used to produce the amphiploid was not available for analysis. However, another accession of this species was analyzed and this showed the presence of four LMW subunits of glutelin (Fig. 3 A, slot 1).

The amphiploid (Fig. 3 A, slot 3) did not exhibit any extra LMW glutelin bands over those present in Chinese Spring. Unexpectedly, it lacked two (B3, B4) of the four labelled LMW glutenin bands known to be controlled by chromosome arm *1BS* in Chinese Spring. This stock, however, possessed a HMW glutelin band (E1) in addition to those present in Chinese Spring. One-dimensional SDS-PAGE patterns of unreduced protein extracts from this seed also revealed a lack of the gliadin bands (B5, B6) controlled by the *Gli-B1* locus in Chinese Spring, but it contained three extra gliadin bands E4, E5, and E6 (Fig. 3 B, slot 3). Four other seeds from this amphiploid stock were analyzed and three of them exhibited gliadin bands B5 and B6, while one lacked them (not shown). Since the frequency of seeds lacking gliadins and LMW subunits of glutenin bands of Chinese Spring was high, the parental amphiploid seed used to multiply this stock was probably hemizygous for chromosome arm *1BS*. Consequently, the presence of a band (E2) with similar mobility to a *1BS*-controlled LMW subunit (B1) in these seeds indicated that the E2 band came from *E. elongata*, but this could not be identified in the normal amphiploid seed because of the overlap in mobility.

Since bands E2 and E3 were present in both the substitution lines *1E* (*1B*) (Fig. 3 A, slot 5) and ditelocentric *1ES* (*1B*) (Fig. 3 A, slot 6), which lacked the *1B*-controlled LMW subunits of Chinese Spring, they must be controlled by genes on chromosome arm *1ES*. Additional evidence for the genetic control of band E2 by gene(s) on chromosome arm *1ES* came from observations that substitution stocks *1E* (*1A*) (Fig. 3 A, slot 4), *1E* (*1D*) (Fig. 3 A, slot 7), and *1ES* (*1D*) (Fig. 3 A, slot 8) showed increased staining intensity of band *B1* of Chinese Spring, which had the same mobility as band E2.

This study also showed that a HMW glutelin subunit (E1) was controlled by genes on chromosome arm *1EL*, as this band was not present in *1ES* (*1B*) and *1ES* (*1D*) (Fig. 3 A). On the other hand, the absence of chromosome arm *1EL* did not affect the expression of gliadin bands E4, E5, E6 in these stocks (Fig. 3 B, slots 6, 8) indicating that these are controlled by genes on chromosome arm *1ES*. These results agree with those obtained by Lawrence and Shepherd (1981) for the HMW glutelin subunit.

Secale species

The two-step banding patterns of seed proteins from five cereal rye (*Secale cereale*) cultivars, Imperial, King II, Dakold, Petkus, and South Australian rye, and two wild rye (*Secale montanum*) genotypes, R-15 (Fig. 4 A) and R-42 (Fig. 4 B, slot 7), were analyzed for the presence of LMW glutelin subunits. The two-step gels provided no evidence that any of the rye cultivars produced LMW glutelin bands. Similarly, *S. montanum*, viz., R-42 did not have any LMW bands. However, *S. montanum* R-15 carried one band R1 (Fig. 4 A, slot 4).

The Chinese Spring-*S. montanum* R-15 amphiploid (Fig. 4 B, slot 1) stock did not have band R1, nor was it

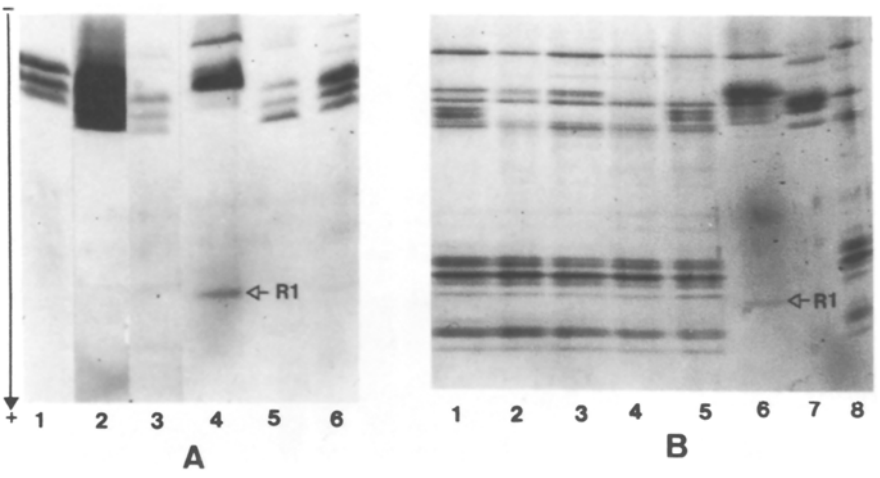

Fig. 4 A and B. Two-step SDS-PAGE patterns of 70% ethanol-extracted seed proteins from rye, wheat controls, and wheat-rye genetic stocks. **A** Cultivated rye (*Secale cereale*) (*1*) Imperial R-95, (*2*) King II, (*3*) Dakold, (*5*) South Australian, (*6*) Petkus R-3, and (*4*) a wild rye (*S. montanum*) genotype R-15. **B** (*1*) Chinese Spring (CS)-*S. montanum* amphiploid ($AABBDDR^mR^m$), addition lines (*2*) CS+$1R^m$, (*3*) CS+$1R^mS$, (*4*) CS+$2R^m$, (*5*) CS+$6R^m$, (*6*) *S. montanum* R-15, (*7*) *S. montanum* R-42, and (*8*) Chinese Spring

present in any of the addition lines involving chromosomes $1R^m$ (Fig. 4B, slot 2), $1R^mS$ (Fig. 4B, slot 3), $2R^m$ (Fig. 4B, slot 4), or $6R^m$ (Fig. 4B, slot 5). The genes controlling band R1 might be suppressed in a Chinese Spring wheat background or, alternatively, the *S. montanum* genotype involved in producing the amphiploid and addition lines did not carry this band. Also, it is possible that the R1 band might not be synthesized in sufficient amount to be visualized in a wheat background.

Discussion

The genes controlling the LMW subunits of glutelin in wheat and species related to wheat have been located on the group 1 homoeologous chromosomes of the Triticeae. In particular, chromosomes *1A*, *1B*, *1D* in hexaploid wheat (AABBDD), chromosome $1S^l$ in *T. longissimum* (S^lS^l), chromosome *1U* in *T. umbellulatum* (UU), and chromosome *1E* in diploid *E. elongata* (EE) all carry genes for LMW glutelin subunits. Although the rye cultivars investigated did not exhibit any LMW subunits of glutelin, other evidence suggests that Imperial rye carries a LMW subunit of glutelin that is controlled by chromosome arm *1RS* (Gupta 1989). *Secale montanum* R-15 also possesses a LMW subunit of glutelin but its chromosomal control could not be determined. Gliadins and HMW subunits of glutelin are also controlled by chromosomes $1S^l$ (this study), *1U*, *1R* (Shepherd 1973; Lawrence and Shepherd 1981), and *1E* (Lawrence and Shepherd 1981; this study). These results thus support the homoeologous relationship between the group 1 chromosomes of wheat and $1S^l$ (Netzle and Zeller 1984), *1U* (Athwal and Kimber 1972), *1R* (Shepherd 1973), and *1E* (Dvorak 1980) of these other species, and indicate that all of these chromosomes are probably derived from a common ancestral chromosome.

Evidence from amino acid and nucleotide sequences of HMW glutelin subunits and gliadins has suggested that different genes at the *Glu-1* and *Gli-1* loci were each derived from an ancestral locus through duplication and divergence (see Kreis et al. 1985 for a review; Bartels et al. 1986; Halford et al. 1987). Limited sequence analyses of LMW glutenin subunits (Okita et al. 1985; Kasarda et al. 1988; Colot et al. 1989) have given similar indications for the LMW subunits. The data presented here indicate that duplication or amplification of the ancestral LMW glutelin gene should have occurred very early in the evolution of wheat, since the diploid wheats (Gupta 1989) and related species *Elytrigia elongata* all carry two or more LMW glutelin subunits. The presence of B and C subunits in all the wheats (Gupta and Shepherd 1990) and related species analyzed, their different isoelectric points (Jackson et al. 1983), their ability to recombine with each other (Singh and Shepherd 1988), and their different sizes suggest that they might be encoded by two gene subfamilies.

Recent studies have also shown that allelic variation in glutenin subunits (HMW and LMW both) are associated with differences in technological qualities of wheat flour (see Payne 1987 for a review; Autran et al. 1987; Gupta et al. 1989a). Thus, the glutelin subunits unique to the wild relatives may be used to widen the range of variation in glutenin subunits in wheats and possibly to improve their technological qualities, since HMW subunits of glutelin from *T. thaoudar* and 75-k γ-secalins (glutelins) from rye (*Secale cereale*) have recently been shown to have positive effects on wheat flour quality (Rogers et al. 1989; Gupta et al. 1989b).

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