

# High molecular weight glutenin subunit variation in the *Sitopsis* section of *Aegilops*. Implications for the origin of the B genome of wheat

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Variation in high molecular weight (HMW) glutenin subunit composition among *Aegilops* species of the *Sitopsis* section, namely *Ae. bicornis*, *Ae. longissima*, *Ae. sharonensis*, *Ae. searsii* and *Ae. speltoides* was investigated using one-dimensional, as well as two step one-dimensional (2S-1D), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The presence of three glutenin bands in several accessions of *Ae. longissima* and *Ae. sharonensis* seems to be evidence for the existence of at least three active genes, called *x*, *y* and *z*, controlling the synthesis of the high molecular weight glutenins in the genome S of *Aegilops*. From comparisons between the glutenin patterns found in the species analysed and those bands observed for the B genome of hexaploid wheat, it can be concluded that *Ae. bicornis*, *Ae. longissima* and *Ae. sharonensis* are unlikely donors of the B genome, mainly because of the presence of specific glutenin markers that were absent in wheat. *Ae. searsii* and *Ae. speltoides* showed glutenin subunits that moved within the range of those of B, but *Ae. speltoides* is the only species that could explain the variability for HMW subunits previously described for the B genome of wheat, and therefore it cannot be excluded as a possible donor of this genome.

## INTRODUCTION

It has been generally accepted that the A genome of both tetraploid and hexaploid wheats is derived from *Triticum monococcum* and the D genome of hexaploids from *Aegilops squarrosa*. However, the source of the B genome remains unresolved. Thus, karyotypic analysis, chromosome pairing and banding patterns, comparative studies of monomorphic protein markers, restriction patterns of chloroplast and mitochondrial DNA, and morphological characteristics, have been used as criteria for determining the donor species of the B genome (see Kerby and Kuspira, 1987 for a review).

The high molecular weight (HMW) glutenins of wheat have been extensively analysed over recent years, and much progress has been made in characterizing glutenin subunits both genetically and biochemically, mainly due to their relationship with breadmaking quality in hexaploid wheat (see Law and Payne, 1983; Payne, 1987). In contrast, HMW glutenins have not been well characterized in species related to wheat, in spite of their much wider range of genetic variability for grain proteins.

This variation can be used not only for genetic improvement but also in evolutionary studies (Feldman *et al.*, 1986).

The aim of this work is to analyse the variability for the HMW glutenin subunits in several accessions of *Aegilops* species included in the *Sitopsis* section, probable donors of the B genome.

## MATERIAL AND METHODS

The seeds analysed in the survey of variation for high molecular weight (HMW) subunits included eleven accessions of *Ae. bicornis* (four from Egypt, one from Israel and six from unknown origin), twenty six accessions of *Ae. longissima* (twenty from Israel, two from Jordan and four from unknown origin), four accessions of *Ae. searsii* (one from Syria and three from unknown origin), seven accessions of *Ae. sharonensis* (four from Israel and three from unknown origin), forty one accessions of *Ae. speltoides* (one from Israel, seven from Iraq, fifteen from Israel, twelve from Turkey and six from unknown origin) and fourteen hexaploid wheat cultivars usually employed as

standards for HMW glutenins (Payne and Lawrence, 1983).

At least ten different seeds from each accession were studied by SDS-PAGE.

Unreduced proteins were extracted from endosperm halves of single kernels using sample buffer without 2-mercapto-ethanol (2-ME) and separated in 10-12 per cent polyacrylamide gel electrophoresis as described by Payne *et al.* (1980). The unreduced extracts were later reduced with one drop of 2-ME and subjected to further SDS-PAGE 10-12 per cent gel in order to observe the high molecular weight proteins.

The same unreduced extracts were also analysed using the two step-one dimensional (2S-1D) method described by Singh and Shepherd (1988), with some modifications. In the first step 400  $\mu$ l of each extract was run for about 1 hour in separate gel tubes (5 mm thick), instead of several slots in the same slab, in normal SDS-PAGE, and the top 1 cm portion was cut and transferred into sample buffer with 2-ME for reduction. Subsequently these reduced protein samples were run as described above for reduced extracts.

The bread wheat varieties "Danchi" and "Hope" were used as standards in all electrophoresis because their high molecular weight subunits covered the migration range of HMW glutenins rather well. The number assignments of wheat bands was carried out according to Payne and Lawrence (1983).

In all cases the molecular weight markers used were the Sigma MW-SDS-200 kit that includes myosin (205,000),  $\beta$ -galactosidase (116,000), phosphorylase B (97,400), bovine albumin (66,000), egg albumin (45,000) and carbonic anhydrase (29,000).

## RESULTS

The HMW glutenins are important components of the total seed storage proteins. This fraction forms large disulphide linked aggregates that can be broken into their component subunits (80-140 kd) by treatment with an agent that breaks disulphide bonds, such as 2-ME, and an agent which disrupts, either directly or indirectly, hydrophobic interactions and hydrogen bonds, such as the anionic detergent (see Bietz and Wall, 1972; Kasarda *et al.*, 1976; Ewart, 1988). Thus, these subunits are not detectable by electrophoresis when the proteins samples were fractioned by SDS-PAGE without prior reduction with 2-ME since the aggregates could not penetrate the pores of the polyacrylamide gel. Conversely, proteins that did not form aggregates were not present in the second step and were not retained on the top of the gel. These properties are typical of glutenin proteins of wheat (see Waines and Payne, 1987) and it would be assumed that the HMW proteins studied in the following *Aegilops* species are equivalent to those glutenin subunits of wheat.

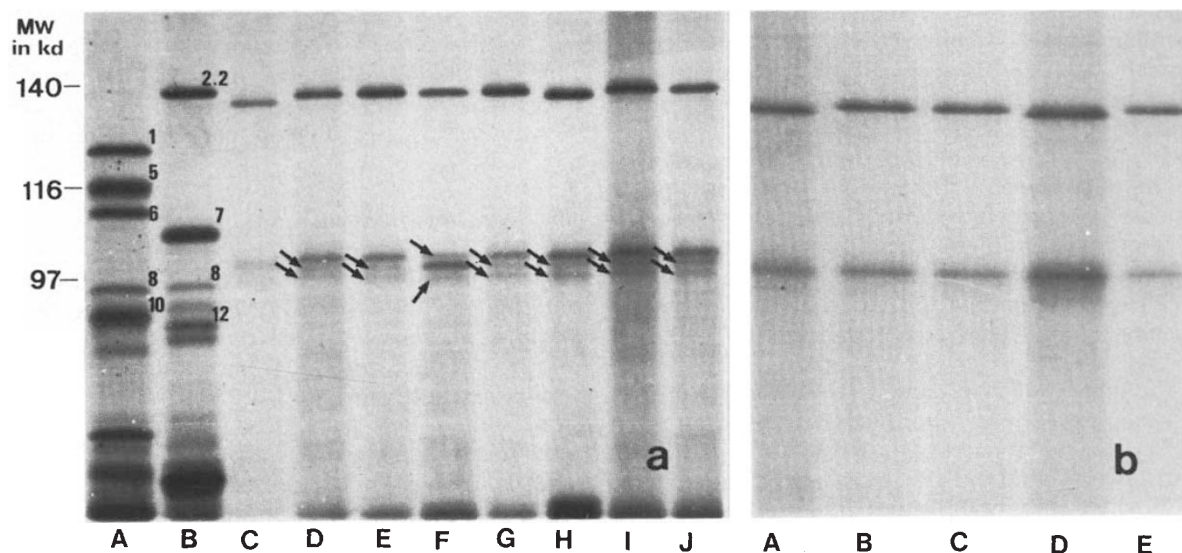
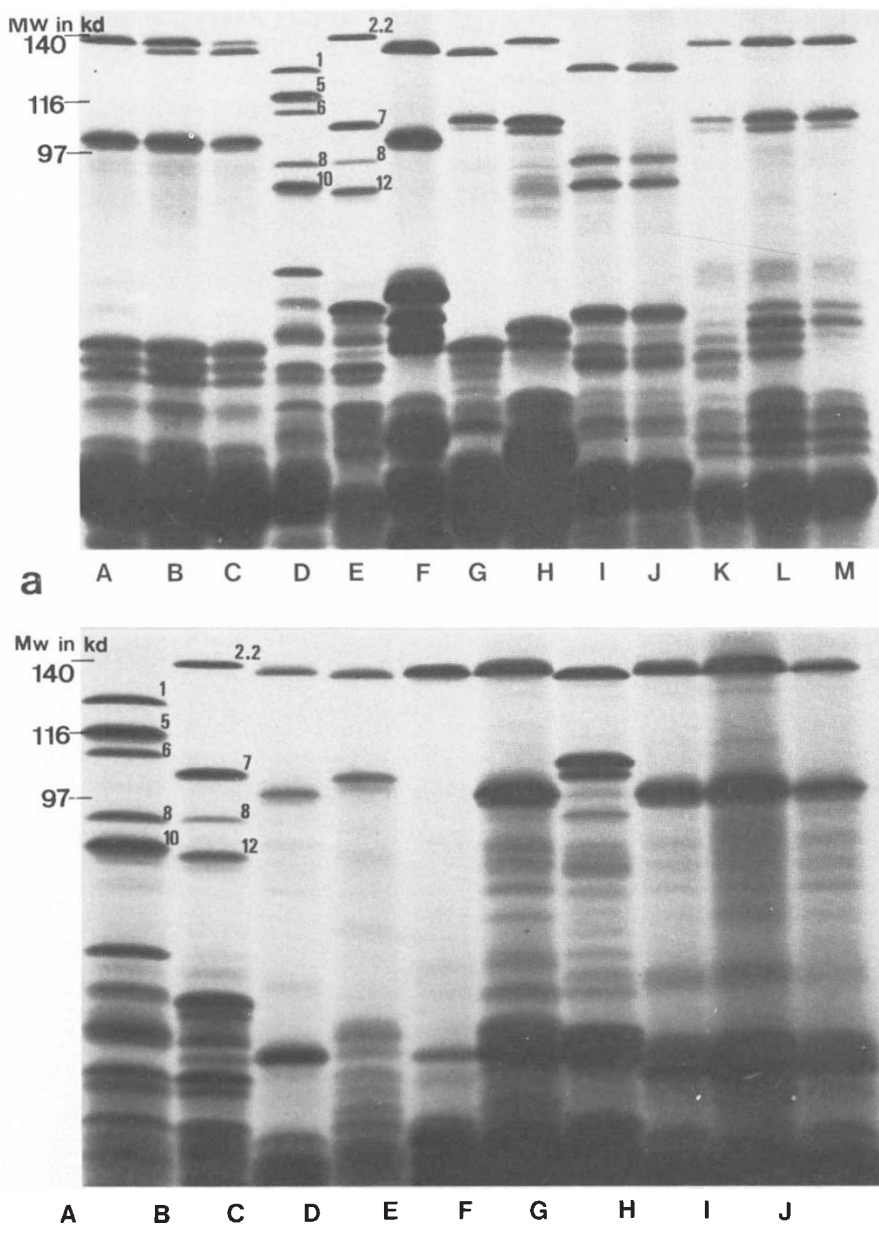


Figure 1 SDS-PAGE migration patterns of HMW glutenins extracted from the samples of *Ae. bicornis*. (a) Reduced total seed proteins. A. *cv.* Hope, B. *cv.* Danchi, C-J. accessions 2, 3, 4, 7, 6, 5, 8, and 9 of *Ae. bicornis*. (b) 2S-1D SDS-PAGE. A-E. accessions 2, 3, 4, 5, and 6 of *Ae. bicornis*. Arrows indicate minor bands that disappeared when the 2S-1D method is employed.



**Figure 2** SDS-PAGE migration patterns of HMW glutenins from several accessions of *Ae. longissima* and *Ae. sharonensis*. (a) *Ae. longissima* A-C. extracts from accession 2, D. *cv.* Hope, E. *cv.* Danchi, F-J. accessions 8, 10, 24, 25 and 26, K-M. extracts from different seeds of accession 11. (b) *Ae. sharonensis* A. *cv.* Hope, B. *cv.* Danchi, C-G. accessions 1, 2, 3, 4, 5., H-J. several extracts of accession 6.

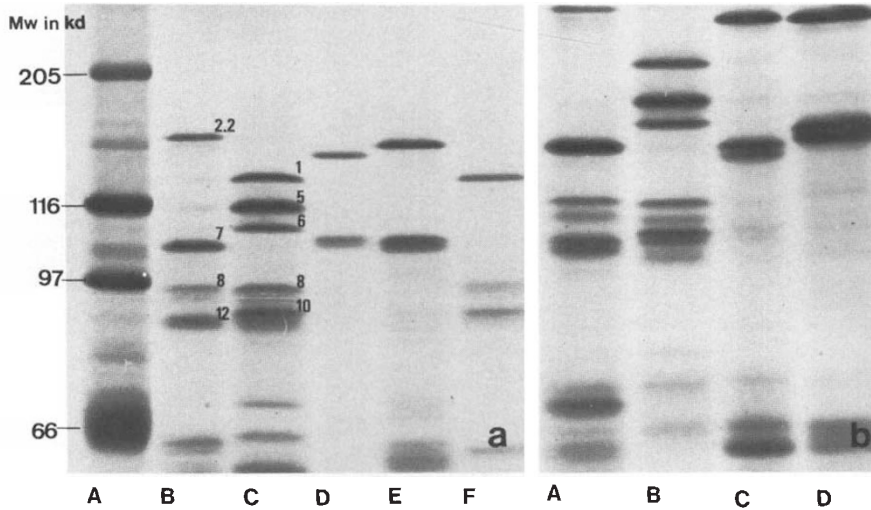
The eleven accessions analysed in *Ae. bicornis* showed a very similar pattern formed by two different electrophoretic moving zones. The slow-moving zone was represented by only one band whose migration was equal to or slightly faster than 2.2 of *cv.* Danchi. The fast-moving zone was composed of one major band and two close minor

bands located between bands 7 and 8 of *cv.* Danchi (fig. 1(a)). However, only the major band was retained when the 2S-1D method was employed (fig. 1(b)).

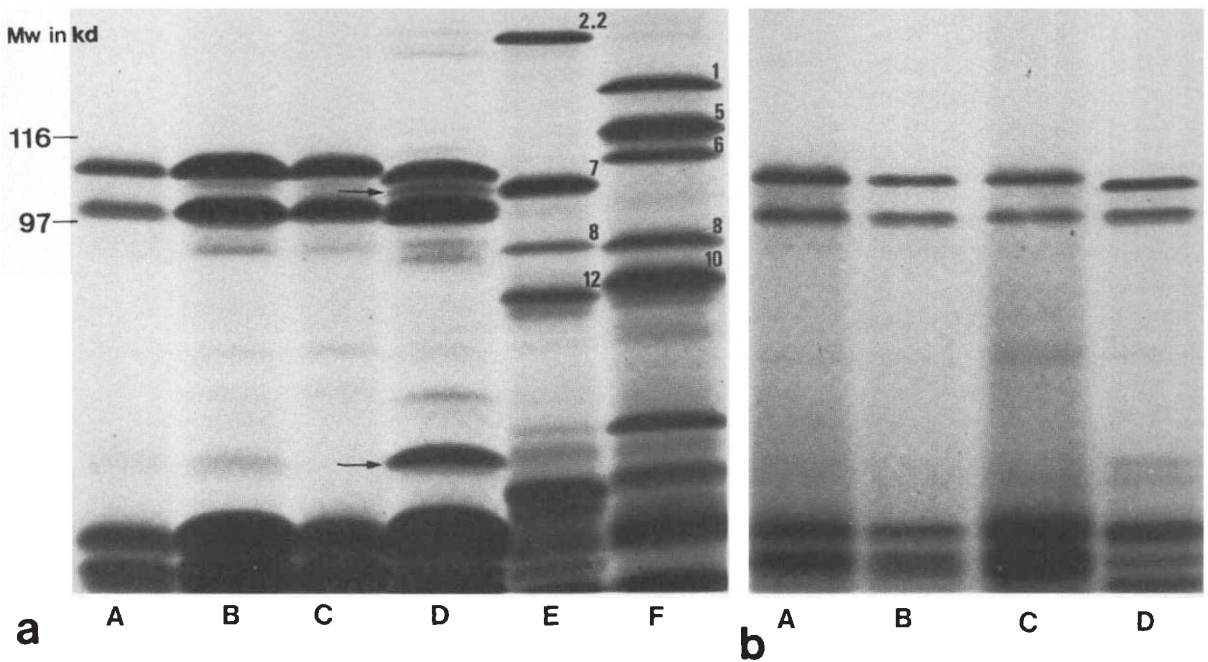
The patterns observed in *Ae. longissima* were more variable. All accessions showed two migrating zones, the slower located between bands 2.2

and 1 of *cv.* Danchi and Hope, respectively. In all accessions this zone was represented by only one band except accession 2 that showed two patterns, one with only one band and the other with two bands (see fig. 2(a), slots A, B and C). The faster-moving zone was composed of two subunits situ-

ated between bands 7 and 8 of *cv.* Danchi, except accessions 25 and 26 that showed two more widely separated bands, one with similar mobility to band 8 and the other at the mobility of band 12 of *cv.* Danchi. Accessions 2 and 8 showed only one wide band for the faster moving zone, Multiple minor



**Figure 3** 2S-1D SDS-PAGE of *Ae. longissima* and *Ae. sharonensis*. (a) *Ae. longissima* A. molecular weight markers, B. *cv.* Danchi, C. *cv.* Hope, D. accession 10, E. accession 11, F. accession 25. (b) *Ae. sharonensis* A. *cv.* Danchi, B. *cv.* Hope, C. accession 4, D. accession 5.



**Figure 4** SDS-PAGE migration patterns of HMW glutenins extracted from samples of *Ae. searsii*, (a) Reduced total seed proteins. A-D. accessions 2, 3, 4 and 5, E. *cv.* Danchi, F. *cv.* Hope. (b) 2S-1D SDS-PAGE. A-D. accessions 2, 3, 4, and 5. Arrows indicate bands that disappeared when the 2S-1D method was employed.



bands have also been observed in several accessions, but these bands have not been considered in this study (fig. 2(a)).

In *Ae. sharonensis* all accessions showed two zones of migration, one of them located very closed to band 2.2 of *cv. Danchi* as in *Ae. bicornis* and the other between bands 7 and 8, except accession 3 that did not show any band for the faster moving zone (fig. 2(b), slot E). As in *Ae. longissima*,

accessions 4 and 5 showed a double band for the faster zone (fig. 2(b)).

In both species these double bands observed for the faster moving zone also appeared when the 2S-1D method was employed (fig. 3), indicating that they form disulphide linked aggregates.

In *Ae. searsii* a very constant pattern formed by two subunits was found, one located between bands 6 and 7 of wheat and other between bands

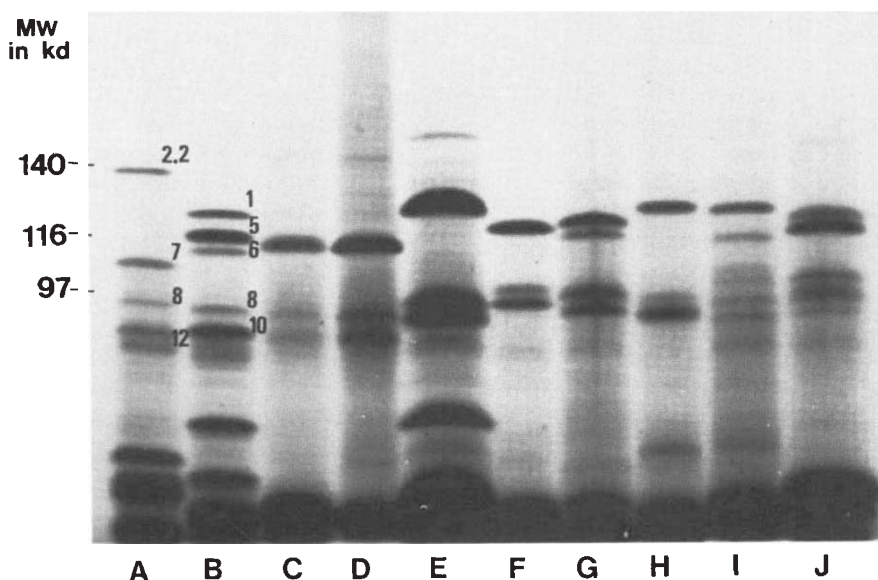


Figure 5 SDS-PAGE patterns of total seed reduced proteins from several accessions of *Ae. speltoides*. A. *cv. Danchi*, B. *cv. Hope*, C-J. accessions 5, 2, 4, 3, 1, 20, 16, and 17.

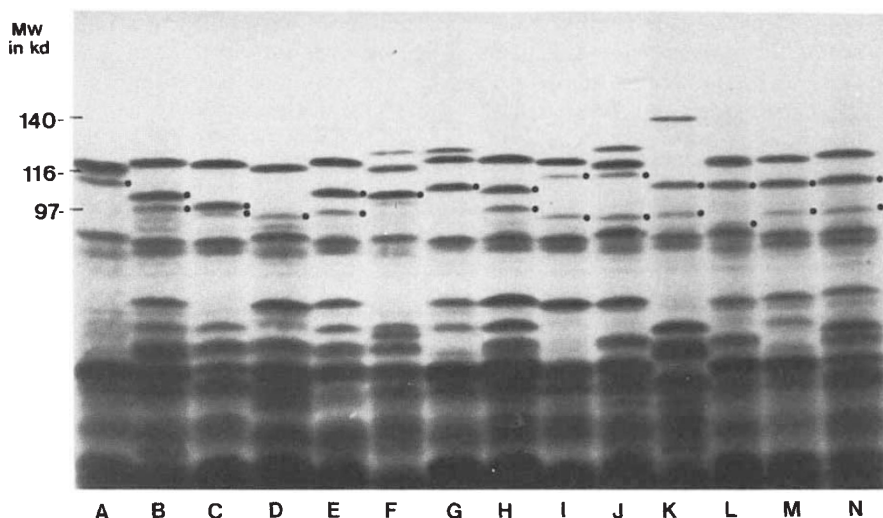


Figure 6 SDS-PAGE patterns of total seed reduced proteins from several wheat cultivars normally used as standards. A. Zorba, B. Sappo, C. Sandown, D. Serbian, E. Lancota, F. Federation, G. Flinor, H. Frondoso, I. Hobbit, J. Hope, K. Danchi, L. Bezostaya-I, M. Champlein, N. Chinese Spring. Fill points indicate bands of the B genome.

7 and 8 of *cv.* Danchi (fig. 4(a)). It is worth mentioning that in accession 5 appeared an extra thin band between the two major subunits, (fig. 4(a), slot D) but that band could not be considered as a glutenin protein because it disappeared when the 2S-1D method was employed (fig. 4(b)).

As expected *Ae. speltoides* showed the most variable patterns of the species analysed here. This variability could be a reflection of the allogamous reproductive system of this taxon. In all accessions analysed, a great number of patterns formed by several subunits were found, and no one specific mobility zone could be determined, but even so, all bands were located between or near bands 1 and 10 of *T. aestivum* (see fig. 5 and 6).

In *Ae. longissima* and *Ae. speltoides*, where the number of bands observed were sufficiently high, a more detailed analysis was made in order to determine the percentage of subunits with the same electrophoretic mobility that those described for the B genome of wheat by Payne and Lawrence (1983). All accessions of *Ae. longissima* and two seeds of each accessions of *Ae. speltoides* were compared with glutenin subunits of standards. In *Ae. longissima* 27 of the total 58 bands (46.55%) and 129 of the total 234 bands (55.13 per cent) in *Ae. speltoides* showed the same mobility as subunits of the B genome.

## DISCUSSION

Numerous experiments have been undertaken to establish the phylogeny of the polyploid wheats. Although it is accepted that A and D genomes are derived from those of *T. monococcum* and *Ae. squarrosa* respectively, evidence about the origin of the B genome of *T. turgidum* and *T. aestivum* is still contradictory (see Kerby and Kuspira, 1988).

Comparative analysis of HMW glutenin subunits could provide additional information for identification of the putative parental species of the B genome. These proteins are the products of the activity of what have been termed complex loci or gene clusters located on homoeologous group 1 in *Triticum* and related species (Bietz *et al.*, 1975; Payne *et al.*, 1980, 1982; Lawrence and Shepherd, 1981; Galili and Feldman, 1983; Levy *et al.*, 1985).

Generally, in all species studied, these clusters contain two genes, namely *x* controlling the subunit that shows the slower electrophoretic mobility and *y* controlling the subunit with faster electrophoretic mobility (Forde *et al.*, 1985; Harberd *et al.*, 1986; Waines and Payne, 1987). In some cases,

two or more bands were observed for the faster moving zone. These subunits appeared as a closely migrating doublet, that always co-migrated and were formed by a wide band (the slowest) and a faint band (the fastest) in most of the cases (see slots G, H, K, L, M in fig. 2(a) and F, G in fig. 2(b)).

It has been suggested by Holt *et al.* (1981) that these double bands are either products of the same gene or, alternatively, products of two closely linked and duplicated genes. This assumption is supported by the similarities found for their mobility in two-dimensional electrophoresis (Holt *et al.*, 1981), amino acid composition and patterns after protease digestion (Galili and Feldman, 1985). The data of tetraploid wheats reported by Levy *et al.* (1988) strongly indicate that such bands are controlled by a single gene, since in all electrophoretic patterns the two subunits were either present or absent and they were always of very similar mobility. If they were encoded by two different genes their allelic variation may not always result in such an electrophoretic pattern. As pointed out by Levy *et al.* (1988) there are several molecular mechanisms to produce two polypeptides from a single gene; one of them could imply proteolytic activity either during endosperm development or protein extraction (Holt *et al.*, 1981; Harberd *et al.*, 1986; Waines and Payne, 1987). This last mechanism could be responsible for faster moving double bands observed in several tetraploid species of *Aegilops* (Vazquez *et al.*, unpublished). In that case the fastest subunit of the doublet disappeared in all accessions in which the 2S-1D method was employed. This was explained by suggesting that the modified polypeptides (proceeding from proteolytic activity) did not form disulphide-linked aggregates and therefore were eliminated in the first step. This seems to be the case for the minor bands found in *Ae. bicornis* (fig. 1(a), 1(b)) but not for those observed in *Ae. longissima* and *Ae. sharonensis*, where neither band of the doublets disappeared using the two steps (fig. 2 and 3). In addition, accessions 25 and 26 of *Ae. longissima* showed two well separated bands that could not be classified as doublets (see slots I, J in fig. 2(a) and slot F in fig. 3(a)), and similar results have been also observed in *Ae. uniaristata* (Vazquez *et al.*, unpublished).

The appearance of three bands could be also explained by the existence of heterozygous plants for glutenin loci. Consequently, plants of different accessions showing three bands were self-pollinated and the progeny of each plant was analysed by SDS-PAGE. In all cases the electrophoretic pattern was the same as that observed in the paren-

tals, except in accession 2 in which segregation for the slow moving zone was found. Therefore there is good evidence for assuming that in *Ae. longissima* and, probably, in *Ae. sharonensis* there must be at least a third gene, called *z*, for HMW glutenins, although further genetic analysis should be made in order to confirm this hypothesis. The presence of more than three bands in *Ae. speltoides* could be explained by the existence of heterozygous plants, since *Ae. speltoides* is an allogamous species.

The variation in the amount and type of grain proteins is the main factor responsible for determining the differences in breadmaking quality and nutritional properties of flours. The unique cohesive-elastic properties of doughs made from flour of hexaploid wheat result primarily from the properties of the gluten proteins (Krull and Wall, 1969; Kasarda *et al.*, 1971), and there is strong evidence that the presence of specific HMW glutenin subunits is correlated positively with improved breadmaking quality (see Payne, 1987). These findings indicate the importance of determining the genetic variation of genes controlling glutenins in wheat and its relatives. The new variation found in the *Aegilops* species studied in this work could be a valuable source for improving breadmaking quality in wheat, by transferring new subunits and/or increasing the number of subunits by using those species, such as *Ae. longissima* and *Ae. sharonensis*, in which the three supposed genes are actively expressed.

The comparisons between the electrophoretic patterns found in *Aegilops* species of the *Sitopsis* section seem to indicate that *Ae. bicornis* can be excluded as the donor of the B genome of wheat. This species showed a very constant pattern bearing one band with a very similar mobility to that of band 2.2 of *cv.* Danchi in all accessions (fig. 1); in addition the persistency of that pattern is not in accordance with the variability found for glutenins in the B genome (fig. 6). Similar conclusions have been reached by Kerby and Kuspira (1988) from karyotypic studies, and by Natarajan and Sarma (1974) from comparisons of chromosome banding patterns.

*Ae. longissima* and *Ae. sharonensis* showed more variable patterns, but again both species consistently show a band with similar mobility to that of band 2.2 of Danchi, absent from the B genome. In addition, it seems clear that *Ae. longissima* and *Ae. sharonensis* have three genes for the high molecular weight glutenins, whereas for the B genome only two copies of such genes have been described (Payne *et al.*, 1982). This result, although

indicative, could not be a reason for excluding these species as donors of the B genome because plants or accessions with null alleles might be implicated in the evolution of wheat, as has been demonstrated for the A genome of *T. monococcum* (Waines and Payne, 1987).

*Ae. sharonensis* does not show the same karyotypic characteristics of the B genome (Kerby and Kuspira, 1988); however, Natarajan and Sarma (1974) indicated that the distribution of C-heterochromatin in the chromosomes of *Ae. sharonensis* is very similar to that observed for chromosomes of the B genome, whereas *Ae. longissima* does not show such a C-banding pattern. By contrast, Ogihara and Tsunewaki (1982) and Tsunewaki and Ogihara (1983) found that the restriction patterns of chloroplast DNA from *T. turgidum* was identical to that of *Ae. longissima*. Konarev *et al.*, (1976, 1979), and Konarev (1983) showed that some albumins and gliadins in *Ae. longissima* and *Ae. searsii* were identical both to one another and to those controlled by the B genome in polyploid wheats.

The HMW glutenins observed in *Ae. searsii* are in the range of mobility found for the subunits of the B genome (between bands 5 and 10). However all accessions analysed showed similar patterns with a very constant location and little variation (see fig. 4). By contrast the B genome is the most variable for HMW glutenins in wheat. Although the number of accessions analysed in this work is very limited, the protein patterns observed in *Ae. searsii* do not clearly resemble the glutenin subunits of the B genome of wheat. This conclusion is in agreement with that suggested by Chen and Gill (1983) from the C-banding patterns of *Ae. searsii* chromosomes. However, Feldman and Kislev (1977) have suggested that *Ae. searsii* is the probable donor of the B genome since it is the only species in the *Sitopsis* section that has a sympatric distribution with both *T. turgidum* and *T. monococcum*, and its satellite chromosomes show the same morphology as that of chromosomes 1B and 6B of *T. aestivum* (Feldman, 1978). The karyotypic analysis carried out by Kerby and Kuspira (1988) seems to indicate the same conclusions.

*Ae. speltoides* was the most variable species analysed in this work and should not be excluded as the donor of the B genome. There were a great number of plants that showed glutenins with an electrophoretic mobility within the range of the HMW subunits for the B genome, and its variability could explain that observed in wheat. In fact, many of those bands of *Ae. speltoides* (55.13 per cent) have the same electrophoretic mobility as the



fourteen subunits of the B genome analysed in the standards wheats. Probably, the mobility of another subunit encoded by chromosome 1B (see Vallega and Waines, 1987; McIntosh, 1988) might coincide with the mobility of any other of *Ae. speltoides* due to the variation found in this species. Many workers have arrived at this conclusion studying morphological characteristics (Sarkar and Stebbins, 1956), satellite chromosomes (Riley *et al.* 1958) cytoplasmic proteins (Barhman *et al.* 1988) chromosome banding (Chen and Gill, 1983), and amount of nuclear DNA (Rees, 1963; Rees and Walters, 1965) whereas many others have excluded it using the same type of analysis (Waines and Kimber, 1973; Natarajan and Sarma, 1974; Gill and Kimber, 1974; Hadlaczky and Belea, 1975; Iodansky *et al.*, 1978; Kerby and Kuspira, 1988).

Probably the contradictory results obtained from different studies could be explained because each type of experiment can only provide limited information. Thus chromosome morphology and banding patterns cannot be taken as an accurate methodology because in many cases chromosome morphology may have changed by chromosome rearrangements, and usually there is an extensive C-banding polymorphism, not only between different species, but also within a given species, which makes comparison difficult. In the same way, protein and isozyme studies could be very useful, but only a few loci are usually available for analysis. From the results obtained from different sources it seems clear that the B genome donor might be a species with as high a level of polymorphism as *Ae. speltoides*, and the analysis of a great number of accessions would be required to identify it unequivocally.

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