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Chromosomal location of gliadin coding genes in *T. aestivum* ssp. *spelta* and evidence on the lack of components controlled by *Gli-2* loci in wheat aneuploids

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Summary. Electrophoretical analyses of the gliadin fraction extracted from seeds of the intervarietal substitution lines of *T. aestivum* ssp. *spelta* in the *T. aestivum* ssp. *vulgare* cv 'Chinese Spring' for the homoeologous groups 1 and 6 and substitution lines of 6D chromosome of 'Chinese Spring' in the durum wheat cv 'Langdon' allowed the identification of seeds without gliadin proteins controlled by genes on chromosome 6A and 6B. A gliadin component of 'Chinese Spring', not previously assigned to any specific chromosome, is controlled by chromosome 6D in the 6D (6A) and 6D (6B) disomic substitution lines of 'Langdon'. Additional genes controlling the synthesis of this component may be present on other chromosomes, very likely 6A and 6B, since the analysis of the 'Chinese Spring' compensating nullisomic-tetrasomics involving the 6D chromosome does not show the loss of this component or any apparent change in staining intensity. Chromosomal location data and two-dimensional gliadin maps reveal close homologies between the two hexaploid wheats, 'Chinese Spring' (*T. aestivum* ssp. *vulgare*) and *T. aestivum* ssp. *spelta*, belonging to different subspecies in the hexaploid group of genomic formula AABBDD. The comparison of gliadin electrophoretic patterns aiding in the identification of evolutionary pathways in wheat is stressed.

Key words: Wheat aneuploids – Null forms – Storage proteins – Gliadins – Evolution

Introduction

The occurrence of bread and durum wheat seeds that lack entire clusters of gliadin and glutenin components has recently been reported. Lafiandra et al. (1987a, b) analyzed electrophoretically single seeds of landraces or named cultivars and discovered several lines lacking certain gliadin and glutenin components.

Gliadin proteins are controlled by complex gene families located on the short arms of chromosomes of the homoeologous groups 1 and 6, designated *Gli-A1, Gli-B1, Gli-D1* (*Gli-1* loci), those present on group 1 chromosomes, and *Gli-A2, Gli-B2, Gli-D2* (*Gli-2* loci), those on group 6 chromosomes (Payne et al. 1984b). The high molecular weight (HMW) glutenin components are under control of genes on the long arms of group 1 chromosomes (*Glu-1* loci). Seeds lacking the entire cluster of gliadin components controlled by chromosomes 1A, 1B, 1D and 6A have been identified and named null lines. In most cases, null lines have been found in mixtures with seeds having normal electrophoretical patterns, but seed stocks containing only seeds null for a particular group of gliadin components were also detected (Lafiandra et al. 1987a). Galili and Feldman (1984) have reported the absence of a high molecular weight glutenin subunit in the intervarietal substitution line of chromosome 1D of 'Timstein' in 'Chinese Spring', in spite of the fact that this particular subunit is present in both parents.

Seeds without the entire cluster of gliadin components controlled by chromosomes 6A and 6B, found in materials different from those previously reported, are described in this paper.

Materials and methods

Seeds of the hexaploid wheat *T. aestivum* ssp. *spelta*, the common wheat *T. aestivum* ssp. *vulgare* cv 'Chinese Spring' and the set of substitution lines in which the chromosomes of the homoeologous groups 1 and 6 of ssp. *spelta* replacing homologous pairs of chromosomes of 'Chinese Spring' have been used. The disomic substitution lines for chromosome 6D of 'Chinese

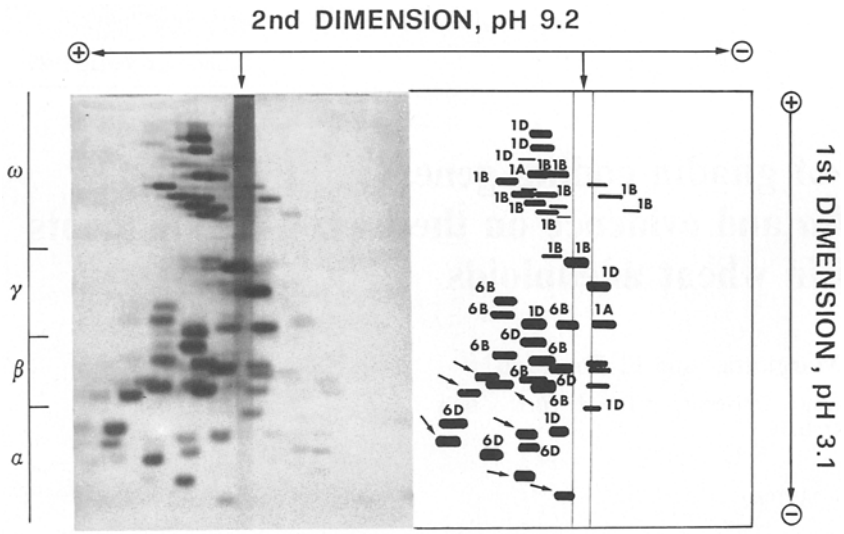

Fig. 1. Chromosomal assignment of gliadin components in *T. aestivum* spp. *spelta*. *Arrows* indicate not assigned components presumably controlled by genes on chromosome 6A

Spring' in the durum wheat cv 'Langdon' were also utilized, along with nullisomic 6A-tetrasomic 6D, and ditelosomic 6BL and 6DL of 'Chinese Spring'.

Gliadins were extracted with 1.5 *M* dimethylformamide and analyzed by two-dimensional electrophoresis at two different pH's according to Lafiandra and Kasarda (1985). Contrary to what was previously reported four, instead of two, gels were cast and run together by using divider plates with the Protean dual 16 cm slab cell (Bio-Rad, Richmond, CA). After the first-dimension electrophoresis and equilibration in the pH 9.2 buffer, pairs of gels were overlapped and run together as a single gel, on a horizontal apparatus, in the second dimension (Lafiandra and Kasarda 1985).

Electrophoretical analyses were performed on half seeds, and the embryo halves were saved. Embryos were germinated and chromosomes were counted using the Feulgen staining technique. Plants were grown from half seeds when necessary.

Results

Chromosomal assignment of gliadin components of the hexaploid wheat T. aestivum ssp. spelta

The two-dimensional separation of the gliadin components extracted from the hexaploid wheat (AABBDD) ssp. *spelta* is reported in Fig. 1. A scheme indicating the chromosomal location for most of the components is shown on the right side of the picture. Forty-five components were clearly identified; in addition, faint spots were also visible on the gel, especially on the cathodic side in the regions of mobilities corresponding to the α and β gliadins. As already reported (Lafiandra et al. 1984), the detection of these components often depends on the amount of protein loaded on the gel. For instance, the two-dimensional separations of gliadins reported in Fig. 4 show, as a consequence of the different amount of proteins loaded, some components on the cathodic side. These correspond to the α region of mobility and are indicated by arrows in Fig. 4d. They are not clearly visible in the remaining three pictures, therefore, they were not considered in the present analysis.

Out of the 45 gliadin components reported in Fig. 1, 32 were assigned to chromosomes 1A, 1B, 1D, 6B and 6D, by analyzing substitution lines of ssp. *spelta* in 'Chinese Spring' for the homoeologous groups 1 and 6. Most of the remaining components, mainly present in the α and β regions (indicated by arrows in Fig. 1) and usually associated with chromosome 6A in bread wheats, were not labelled.

Group 1 chromosomes control the synthesis of ω-gliadins, some of the γ- and two minor components in the α region; but most of the cathodic components in the β region of mobility could also be assigned to this group. Group 6 chromosomes control components with mobilities corresponding to α and β gliadins; a few components found to be controlled by the 6B chromosome were also present in the γ region. These results confirm those already obtained in common wheats 'Cheyenne' and 'Chinese Spring' (Lafiandra et al. 1984).

Chromosomal location data for gliadin components in both ssp. *spelta* and 'Chinese Spring' (Lafiandra et al. 1987a) and direct comparison of the two-dimensional electrophoretical patterns (Fig. 2) indicate the presence of components common to the two wheats. The possibility of running four different samples on different gels under identical conditions, by using divider plates, makes the comparisons easier; Fig. 2 was obtained by superimposing the two gels containing gliadins from ssp. *spelta* and 'Chinese Spring', reported in Fig. 3a and b, respectively. The results are similar to those obtained when 1:1 mixtures of gliadin extracts from both wheats were separated on the same gel (data not shown), but in this latter case, an overloading of the gel is necessary to detect all

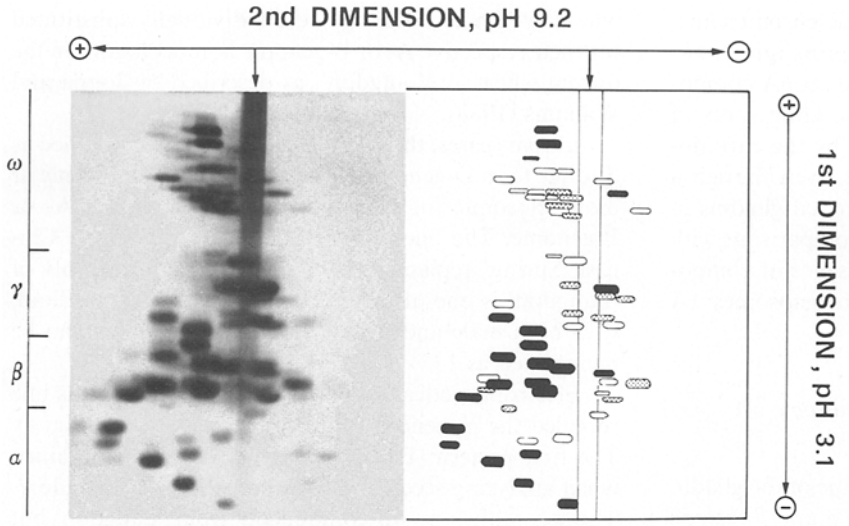

Fig. 2. Superimposed two-dimensional gels of ssp. *spelta* and 'Chinese Spring'. Black spots represent common components of the two wheat. Components of ssp. *spelta* are represented by blank spots, while those of 'Chinese Spring' are reported as dotted spots

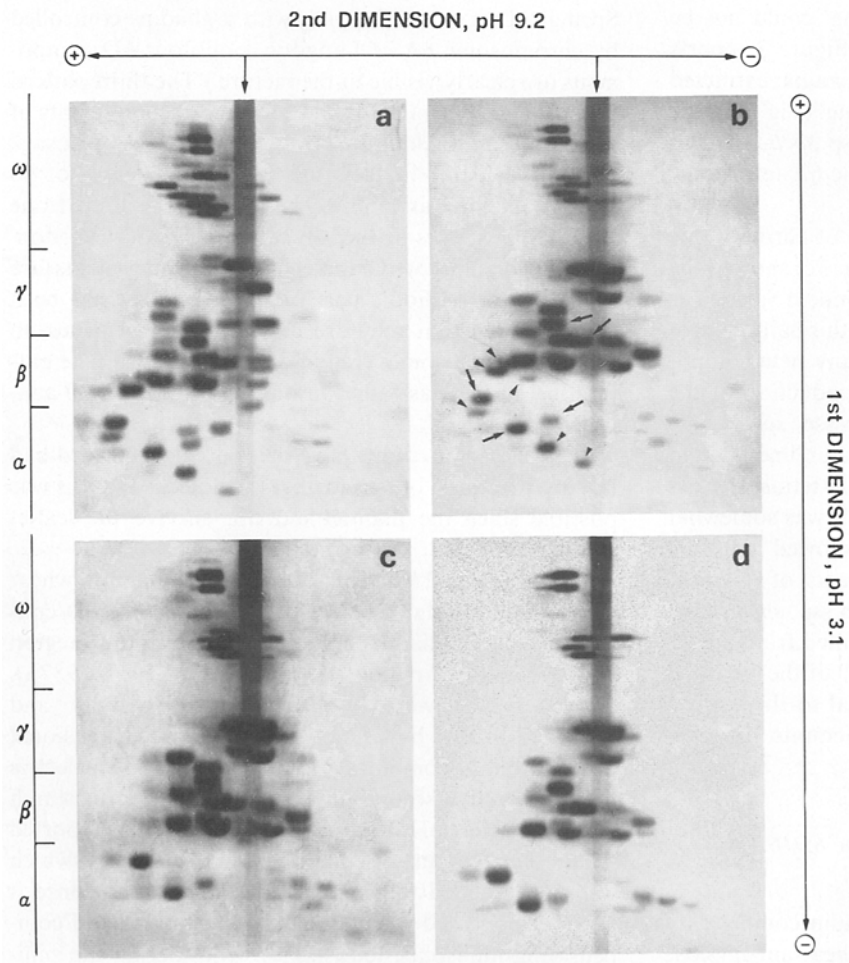

Fig. 3a–d. Two-dimensional separation of gliadin components extracted from ssp. *spelta* **a**, 'Chinese Spring' **b**, substitution line of chromosome 6A of ssp. *spelta* in 'Chinese Spring' **c** and nullisomic 6A-tetrasomic 6D of 'Chinese Spring' **d**. Gliadin components controlled by the chromosomes 6A (*arrowheads*) and 6D (*arrows*) are indicated (Fig. 3b)

components. However, this overloading causes distortion, which makes the interpretation more difficult.

The right side of Fig. 2 shows the components which are common to ssp. *spelta* and 'Chinese Spring'; the common components controlled by chromosome 6D are particularly evident (Fig. 3a and b). Also, most of the components controlled by 1D and 6B chromosomes coincide; three additional components, two controlled by 1D and one by 6B chromosome, were present in the γ region of ssp. *spelta*. Most of the components in the α and β regions

of ssp. *spelta,* not assigned to any specific chromosome, coincide with components of 'Chinese Spring' present in the same regions; in fact, four out of the six 6A components of 'Chinese Spring' have corresponding spots in ssp. *spelta.* The components controlled by the chromosomes 1A and 1B are entirely different, even though a partial overlapping occurs for 1B-controlled gliadins in the ω-region of intermediate mobility. Comparisons with other bread wheats have shown the presence of components similar to those controlled by chromosomes 1A and 1B of ssp. *spelta.*

Analysis of the substitution line of chromosome 6A of ssp. spelta in 'Chinese Spring'

As mentioned in the previous section, some of the gliadin components of ssp. *spelta* present in the α and β regions and having the same two-dimensional mobilities of 6A-encoded components in 'Chinese Spring' could not be assigned to any specific chromosome. Figure 3 reports the two-dimensional separation of gliadins extracted from ssp. *spelta* (Fig. 3a), 'Chinese Spring' (Fig. 3b), the substitution line of chromosome 6A of ssp. *spelta* in 'Chinese Spring' (Fig. 3c) and the nullisomic 6A-tetrasomic 6D of 'Chinese Spring' (Fig. 3d).

The analysis of the substitution line of chromosome 6A of ssp. *spelta* in 'Chinese Spring' (Fig. 3c) showed the absence of 6A gliadin components of 'Chinese Spring', as a logical consequence of the removal of this pair of chromosomes; however, it failed to show any new component, as would be expected for the introduction of the homologous pair of chromosomes from ssp. *spelta.* The two-dimensional pattern of this particular line was, in fact, identical to that of the nullisomic 6A-tetrasomic 6D of 'Chinese Spring' (Fig. 3d). This result was somewhat unexpected, since previous analyses showed that 6A chromosome controls gliadin components of 'Chinese Spring' identical to α-gliadins present in ssp. *spelta,* and no appreciable change should have resulted from analysis of this particular substitution line. All of the analyzed seeds of this line had a pattern identical to the one reported in Fig. 3c, and chromosomal counts have revealed 42 chromosomes.

Analysis of chromosomal substitution line 6D(6B) in the durum wheat cultivar 'Langdon'

A seed without an entire cluster of gliadin components was detected when analyzing durum wheat aneuploids. Chromosomal location of genes coding gliadin components, by using one- and two-dimensional techniques, has been possible in the past few years (Joppa et al. 1983; du Cros et al. 1983; Lafiandra et al. 1983). This was achieved by using a set of durum wheat aneuploids in which the seven D-genome chromosomes of the common wheat cv 'Chinese Spring' were individually substituted for their respective A- or B-genome homoeologous in the durum wheat cv 'Langdon', as described by Joppa and Williams (1988).

In some cases, the substitution lines are maintained as disomic for a D-genome chromosome and monosomic or monotelosomic for its respective homoeologous A- or B-genome. The line in which chromosome 6D of 'Chinese Spring' replaces the pair of chromosomes 6B of 'Langdon' is one of them. The 6D(6B) line is pistilloid, with both male and female sterility; however, it can be maintained as $13''+1''6D+t'6BS$.

Electrophoretical analyses of single seeds of this line revealed the presence of four different patterns (Fig. 4). The first pattern (Fig. 4a) is identical to that obtained when analyzing seeds of the durum wheat cv 'Langdon'. The second has no 6B components from 'Langdon' but contains the 6D gliadin components from 'Chinese Spring'. (Due to overlapping with α gliadins controlled by chromosome 6A of Langdon, only four 6D components are clearly visible in the picture.) The third pattern (Fig. 4c) has both the 6B and 6D gliadin components of 'Langdon' and 'Chinese Spring', respectively. A dosage effect is evident; in fact, 6D components are not so strongly stained as in Fig. 3b. Finally, the fourth one (Fig. 4d) possesses neither 6B components of 'Langdon' nor 6D gliadins of 'Chinese Spring'. Components are visible in the cathodic part of the gel and it has been demonstrated that they are under control of genes on group 1 chromosomes (Lafiandra et al. 1987a). The pattern in Fig. 4d was found in only 1 seed out of 30 analyzed.

Chromosome counts have revealed that this seed had 28 chromosomes, but no further cytological analysis was possible since the plantlet did not survive the leaflet stage.

Chromosomal location studies in the common wheat cv 'Chinese Spring' revealed that chromosome 6D controls five major gliadin components, three in the α region and two in the β region (Lafiandra et al. 1984, 1987a). Similar results were also achieved by Wrigley and Shepherd (1973) by using a different two-dimensional procedure that combines a first separation in isoelectric focusing with a second-dimension separation in starch gel under acidic pH. Both groups of researchers reported the presence of a major component in the β region, which was not assigned to any specific chromosome, since it never disappeared when analyzing the entire set of compensating nullisomic-tetrasomics. Considering the similarities between the two different electrophoretical procedures (see discussion in Lafiandra et al. 1984) and the position of the spot on the two-dimensional map in Wrigley and Shepherd (1973), it is reasonable to assume that the two groups are dealing with the same component.

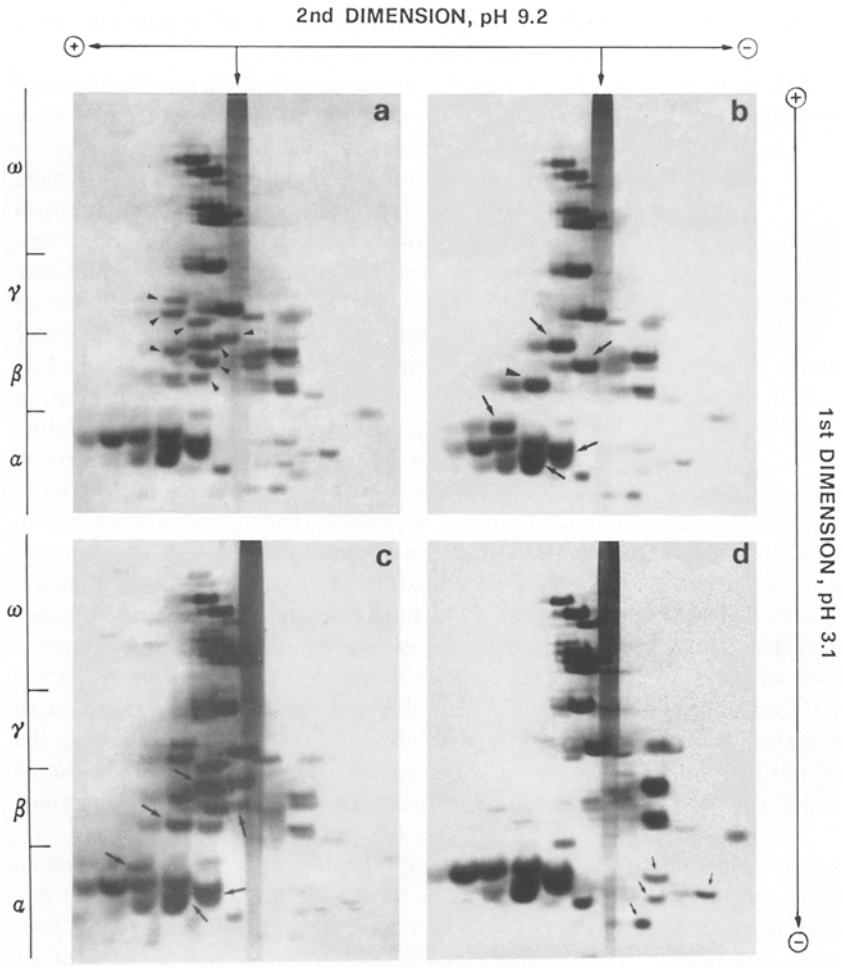

Fig. 4a–d. Two-dimensional separation of gliadins extracted from single seeds of 'Langdon' disomic substitution line 6D (6B). Components controlled by the chromosome 6B of 'Langdon' are indicated in **a**, while those controlled by the chromosome 6D of 'Chinese Spring' are indicated in **b** and **c**. The *arrowhead* in **b** indicates the not previously assigned 6D component. In **d**, only gliadin components controlled by chromosomes 1A, 1B and 6A of 'Langdon' are visible; *small arrows* indicate minor components, appearing clearly only when larger amount of proteins were loaded on the gel

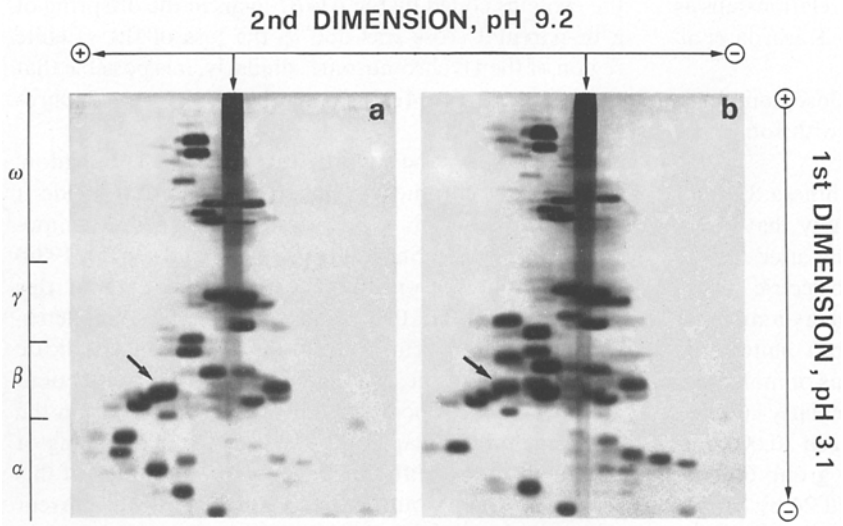

Fig. 5a and b. Two-dimensional separation of gliadin components extracted from nullisomic 6B-tetrasomic 6D **a** and ditelocentric 6DL of 'Chinese Spring' **b**. Component indicated was found to be controlled by the chromosome 6D analyzing the substitution lines 6D (6A) and 6D (6B) of 'Chinese Spring' in 'Langdon'

Results of the analyses of the 6D(6A) and 6D(6B) substitution lines of 'Langdon' showed that the introduction of the 6D chromosome of 'Chinese Spring' into 'Langdon' adds six major components instead of the expected five, three in the α and three in the β region. Comparison of the electrophoretical patterns indicated that the extra component present in the patterns of the substitution line 6D(6B) in Fig. 4b corresponds to the unassigned component. This component is always present in the compensating nullisomic-tetrasomics of the

homoeologous group 6 (see, e.g., the pattern of the nullisomic 6A-tetrasomic 6D in Fig. 3d) and in the 'Chinese Spring' ditelocentric lines 6BL and 6DL (Fig. 5). Any apparent change in intensity of this spot failed to be detected when the different aneuploid stocks of the homoeologous group 6 were considered.

Discussion

Hexaploid wheats (2n = 6x = 42) of genomic formula AABBDD form a species, *T. aestivum*, which includes different subspecies, namely: *compactum, macha, spelta, sphaerococcum, vavilovii* and *vulgare*.

Johnson (1972), using protein electrophoretic patterns of the non-gliadin fraction of the 70% ethanol extracts, was able to demonstrate that all the subspecies in the group have a very uniform profile, which is simulated by the pattern produced by mixing proteins from *T. dicoccum* (AABB) and *Ae. squarrosa* (DD). This result favored the hypothesis that all the *aestivum* subspecies have a monophiletic origin and that cultivated hexaploids evolved from the so-called primitive 'spelta complex' by mutation of a single gene and not by independent crosses between *Ae. squarrosa* and different tetraploids.

The gliadin fraction is a very heterogeneous class of proteins, with a high degree of intraspecific variation. Due to this fact, it has been extensively used in varietal identification and related fields (Wrigley et al. 1982) and assists in establishing wheat evolutionary relationships as stressed by Konarev et al. (1979) and by Kasarda et al. (1984), among others.

Present results cautiously indicate a close homology between *vulgare* and *spelta* in accordance with Johnson's (1972) monophiletic idea.

Substitution lines of ssp. *spelta* in 'Chinese Spring', identical to those used in the present study, have also been analyzed by Brown et al. (1981). They used a two-dimensional electrophoretical system (isoelectric focusing in the first dimension and SDS-polyacrylamide gel electrophoresis in the second dimension) suited for studying subunits of wheat storage proteins of molecular weight greater than 40,000, but not particularly suitable for subunits with molecular weights between 30,000 and 40,000, many of which are controlled by group 6 chromosomes. Two subunits, numbered 20 and 24 by Brown and Flavell (1981), were identified on chromosome 6A of 'Chinese Spring'. Subsequently, Brown et al. (1981), found that the two wheats have identical subunits controlled by this particular chromosome, and other subunits were not detected. However, these authors mention that ssp. *spelta*, along with 'Hope', 'Lutescens' and 'Synthetic', produce some major subunits which are not detectable in the pattern of any of their group 1 and 6 chromosome substitution lines.

Results of the studies aimed at identifying chromosomes which control the synthesis of wheat gliadins have shown that these loci are located on the short arms of the homoeologous group 1 and 6, and extension of these studies to wheat relatives suggests a similar pattern in these materials (Wrigley 1982). The evidence on the similarities between ssp. *spelta* and 'Chinese Spring' also suggests that the unassigned gliadin components of *T. spelta* present in the α and β regions might be under control of genes located on chromosome 6A. The lack of this group of proteins in the corresponding substitution line can be due either to loss of the genes during production of the lines or to their inactivation in the genetic background of 'Chinese Spring' (Brown et al. 1981). On the other hand, it is possible that the initial stock of ssp. *spelta* used in the production of these substitution lines was made by a mixture of seeds with a normal gliadin pattern and seeds not possessing 6A chromosome controlled gliadins, a possibility that should not be overlooked since we have shown that the bread wheat cultivar 'Raeder' is one of these materials (Lafiandra et al. 1987b). The possibility of a translocation involving the short arm of chromosome 6A in ssp. *spelta* should also be considered as a further explanation for the anomalous pattern reported in Fig. 3c.

The situation is quite different in the substitution line 6D(6B) of 'Chinese Spring' in 'Langdon', since four different electrophoretical patterns are present in this line. Payne et al. (1984b) demonstrated that the lack of all the proteins coded by the *Gli-B1* locus in the offspring of a tri-parental cross was due to the loss of the satellite region of the 1B chromosome; similarly, it is possible that the pattern in Fig. 4d is due to the loss of a small chromosomal segment.

The 6D(6A) and 6D(6B) substitution of 'Langdon' with the 6D chromosome of 'Chinese Spring' made it possible to associate a previously unassigned β component (Wrigley and Shepherd 1973; Lafiandra et al. 1984) with this chromosome. The constant presence of this component in all the compensating nullisomic-tetrasomic and ditelocentric lines analyzed would indicate that more than one gene is controlling its production; alternatively, it is possible that the spot observed on the two-dimensional map is the result of the overlapping of different proteins differing in neutral amino acids. If this is the case, they would not be resolved with the current electrophoretical techniques.

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