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 Spring'...
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 α-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.