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Chromosomal location of genes encoding low molecular weight prolamins from rye endosperm

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Summary. A group of proteins with similar Mr, isoelectric points and amino acid composition to those previously described for the low molecular weight prolamins (LMWP) of wheat and barley were isolated from the endosperm of rye (*Secale cereale* L.). Genes controlling four components of this protein group have been assigned to chromosome arm 4RL, through the two-dimensional electrophoretic analysis of *T. aestivum-S. cereale* disomic and ditelosomic addition lines. This observation, together with the previous assignment of LMWP genes in wheat to chromosome groups 4 and 7, is discussed in relation to the proposed 4R/7R chromosomes translocation in *S. cereale.*

Key words: *Secale cereale* – Chromosomal location – Endosperm proteins – 4R/7R translocation

Introduction

The chemistry and genetics of prolamins, the main endosperm storage proteins of species such as wheat, barley and rye, have been extensively investigated in the last few years because they determine to a large extent the nutritional and technological properties of these cereal grains (see Garcia-Olmedo et al. 1982; Shewry and Miflin 1985). The structural homology among the sulphur-rich domains of prolamins, some low molecular weight proteins from cereal endosperm and the 2S globulins from dicots (Kreis et al. 1985) has opened new perspectives about the origin and evolution of these seed proteins and the structural relationships among different endosperm protein groups.

The prolamin preparations of wheat and barley endosperm contain a group of low molecular weight basic components (Mr = 16–20 Kd; pI > 9), which have similar solubility properties, electrophoretic mobility at pH 3.2 and amino acid composition as the major prolamins, although sulphur amino acids are atypically high (see Shewry and Miflin 1985). These proteins have been designated low molecular weight prolamins (LMWP; Salcedo et al. 1979; Aragoncillo et al. 1981). Individual components of this group have been isolated and characterized in wheat (Prada et al. 1982: Cottenet et al. 1984) and barley (Salcedo et al. 1982), and protein fractions enriched in LMWP have been described in rye (Charbonnier et al. 1981). Genes controlling five members of the group have been assigned to chromosomes 7A, 4B and 7D in hexaploid wheat (Salcedo et al. 1980).

Further chemical characterization of LMWP, as well as studies of the chromosomal location of their corresponding structural genes in other species, are desirable for a better definition of this protein family and for a further clarification of their evolutionary relationship with the major prolamins.

We now report the isolation of LMWP from rye endosperm, their identification in two-dimensional protein maps and the chromosomal assignment of genes encoding four members of the group in *Secale cereale.*

Materials and methods

Biological materials

Flour of 65% extraction from *Secale cereale* L., INIA c/171-M, was used for the isolation of rye LMWP. Imperial rye-Chinese Spring wheat and King II rye-Holdfast wheat disomic and ditelosomic addition lines used for the genetic study were the

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gifts of E. R. Sears (University of Missouri, Columbia, USA), S. M. Reader (Plant Breeding Institute, Cambridge, England), F. J. Zeller (Technische Universität München, FRG) and C. Benito (Universidad Complutense, Madrid, Spain).

Protein extraction and gel filtration

The crude 70% ethanol extract from rye endosperm was prepared from delipidated flour (petroleum ether; b.p. 50°–70 °C; 1 × 10 v/w; 1 h) by two sequential extractions with 70% ethanol (10 v/w; 1 h). The ethanol extract was fractionated by gel filtration on Ultrogel AcA-54 as reported by Prada et al. (1982).

Kernels (~30 mg) were extracted with 70% ethanol as previously described (Salcedo et al. 1980).

One- and two-dimensional gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli (1970) and one-dimensional polyacrylamide gel electrophoresis (PAGE) was performed in 0.1 M aluminum lactate buffer (pH 3.2), 3M urea (10% polyacrylamide; 9 × 9 × 0.1 cm gels; 40 V/cm; 1.5 h; 4 °C). Two-dimensional protein maps were obtained by combined electrofocusing (IEF; 5% polyacrylamide, 6M urea; 14 × 0.2 Φ cm gels; ampholines pH 9–11; 35 V/cm; 7 h; sample insertion at pH 9 end) × SDS-PAGE (Laemmli 1970). All gels were stained with Coomassie Brilliant Blue G-250 (Blakesley and Boezi 1977).

The pH gradient in the IEF gels was determined according to Drysdale et al. (1971), and ribonuclease (pI 9.45) and cytochrome c (pI 10.65) were used as testers.

Amino acid analysis

Protein hydrolysis was carried out according to Moore and Stein (1963) and performic acid oxidation as in Hirs (1967). Amino acid analyses were performed as described by Bidlingmeyer et al. (1984).

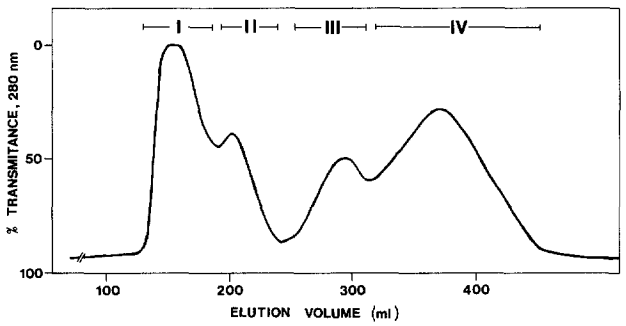

Fig. 1. Gel filtration on Ultrogel AcA-54 of 70% ethanol extract from rye endosperm

Results

Isolation of the LMWP group from rye endosperm and its identification in two-dimensional protein maps

The 70% ethanol extract from rye endosperm was fractionated by gel filtration on Ultrogel AcA-54 (Fig. 1). The fractions obtained (I–IV in Fig. 1) were subjected to SDS-PAGE and PAGE, pH 3.2 (Fig. 2). Fractions I and II corresponded to the main rye prolamins (secalins) and fraction IV contained low-molecular-weight polypeptides that had similar electrophoretic mobilities at acid pH as those of the wheat and barley CM-proteins (Aragoncillo et al. 1981; Paz-Ares et al. 1983).

Components of fraction III had elution volumes on Ultrogel AcA-54, molecular weights (Mr 21–17 Kd) and electrophoretic mobilities in PAGE pH 3.2 that corresponded to those of the previously isolated low-molecular-weight gliadins and hordeins (Aragoncillo et al. 1981; Prada et al. 1982), indicating that this

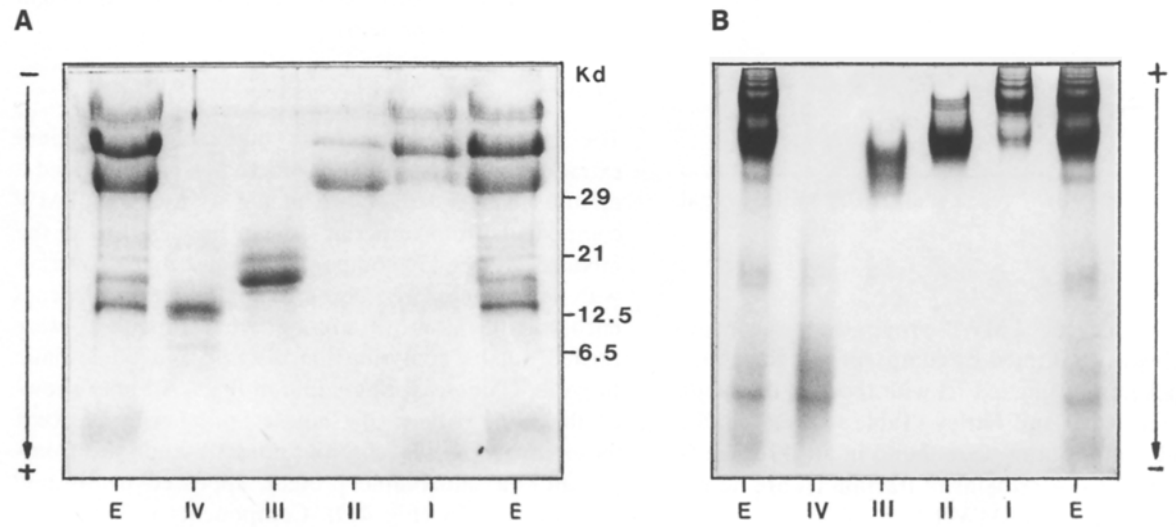

Fig. 2. **A** SDS-PAGE and **B** PAGE, pH 3.2 of the gel filtration fractions (I–IV) indicated in Fig. 1 and of the crude 70% ethanol extract (*E*)

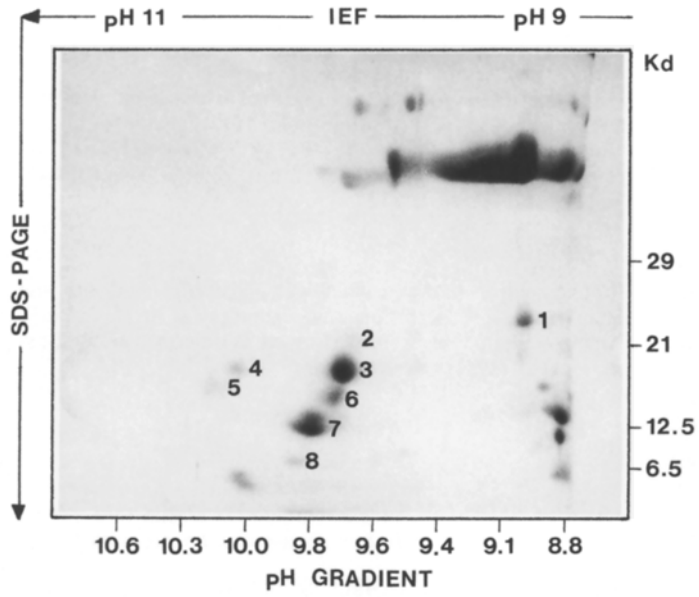

Fig. 3. Two-dimensional protein map (IEF pH 9–11 × SDS-PAGE) of a crude 70% ethanol extract from rye kernels, INIA c/171-M

Table 1. Amino acid composition of fraction III (see Fig. 1) and major low molecular weight prolamins (LMWP) from wheat and barley (mol/100 mol of amino acid analysed)

Amino acid[a]	Fraction III	Wheat[b] LMWG-1	Barley[c] LMWH-1
Lys	0.4	0.3	0.1
His	0.6	0.6	0.2
Arg	2.8	2.4	2.5
Asx	2.6	1.8	2.2
Thr	7.7	7.1	9.6
Ser	6.6	7.5	12.2
Glx	28.5	27.4	26.2
Pro	8.8	11.3	7.0
Gly	6.0	7.5	6.0
Ala	6.2	7.2	7.2
Val	5.2	4.1	4.4
½ Cys	8.3	6.4	7.1
Met	3.5	3.9	3.6
Ile	4.1	4.2	3.3
Leu	4.8	4.1	4.0
Tyr	1.8	1.9	2.3
Phe	2.0	2.3	2.1

[a] Trp was not analysed; [b] Prada et al. 1982; [c] Salcedo et al. 1982

fraction included the LMWP group from rye endosperm. This was confirmed by comparison of the amino acid composition of fraction III with those of the major LMWP from wheat and barley (Table 1). Very similar amino acid compositions were found in all three cases, indicating that major proteins in fraction III are homologous to LMWG-1 and LMWH-1.

Two-dimensional electrophoretic fractionation of LMWP extracted with 70% ethanol from rye kernels was achieved, making use of their high isoelectric points (pI > 9) and low molecular weight (see Fig. 3). The first dimension, IEF (pH 9–11), selected only basic proteins; the second dimension, SDS-PAGE, separated LMW proteins from classical prolamins of pI > 9. The two-dimensional protein map of Fig. 3 was found to be identical for INIA c/171-M rye and for Imperial rye. Components with MW under 25 Kd included in this protein map can be subdivided into two classes: while components 1–6 appeared only in fraction III and were not readily extracted by 0.5 M NaCl, components 7 and 8 appeared exclusively in fraction IV and were quantitatively extracted by salt solutions. It was concluded that proteins represented by spots 1–6 are LMWP, and spots 7 and 8 correspond to low-molecular-weight basic, salt-soluble proteins.

Chromosomal location of genes encoding rye LMWP

The two-dimensional protein maps of 70% ethanol extracts from Imperial rye, Chinese Spring wheat and a mixture of both, are shown in Fig. 4 (A-C). All LMW components from Imperial rye can be identified in the mixture, except for components 2 and 4 that overlap with wheat ones. The chromosomal location of genes encoding the non-overlapping rye components were carried out by analysing the disomic and ditelosomic Imperial-Chinese Spring addition lines. All lines showed the same pattern of Chinese Spring wheat, except those involving rye chromosomes 3R and 4R. Component 7, a salt-soluble protein, appeared in disomic addition line 3R (Fig. 4D). Components 1, 3, 5 and 6 that correspond to LMWP, together with spot 8, another salt-soluble protein, were present in disomic

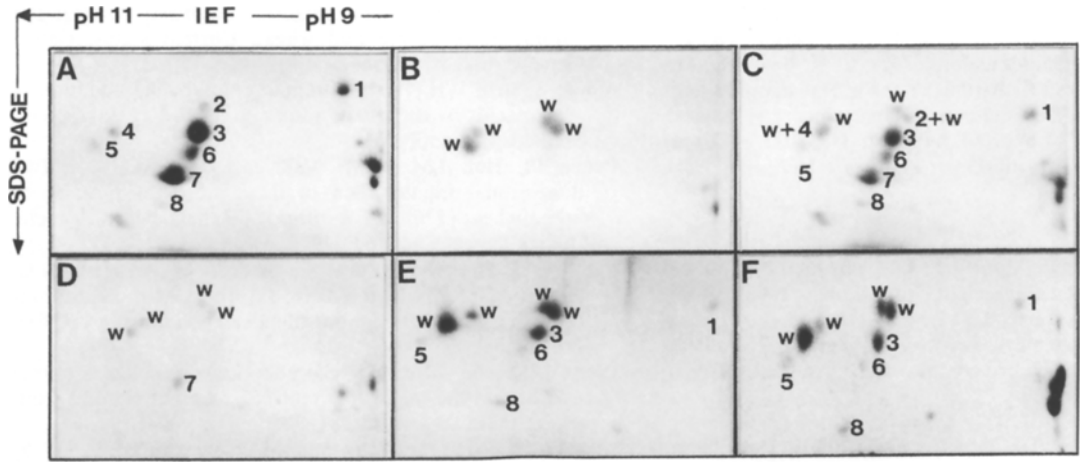

Fig. 4A–F. Two-dimensional electrophoretic analysis of 70% ethanol extracts from kernels of the following samples: **A** *Secale cereale,* cv Imperial; **B** *Triticum aestivum,* cv Chinese Spring; **C** a mixture of Imperial and Chinese Spring extracts; **D** Imperial/Chinese Spring disomic addition line 3R; **E** Imperial/Chinese Spring ditelosomic addition line 4RL; **F** King II/Holdfast ditelosomic addition line 4RL. Only pertinent zones of the two-dimensional maps are shown

addition line 4R (not shown). All these proteins were absent in ditelosomic addition line 4RS and present in the 4RL line (Fig. 4 E).

The chromosomal location of genes that control rye LMWP was further analysed in King II rye-Holdfast wheat addition lines. Similar two-dimensional protein maps to those presented for Imperial rye (Fig. 4 A) and Chinese Spring (Fig. 4 B) were obtained for King II and Holdfast, respectively. Components 1, 3, 5, 6 and 8 only appeared in addition lines 4R (not shown) and 4RL (Fig. 4 F).

Discussion

A group of proteins from rye endosperm that correspond to the LMWP described in wheat and barley (Salcedo et al. 1979, 1980, 1982; Aragoncillo et al. 1981; Prada et al. 1982; Cottenet et al. 1984) were isolated based on the similarity of molecular weights, electrophoretic properties, isoelectric points and amino acid compositions. Chemical characteristics of the LMWP reported here closely resemble those of the A-secalins described by Charbonnier et al. (1981), so both preparations probably include the same main components.

The two-dimensional procedure that was developed to resolve rye LMWP also separates other proteins, presumably basic albumins and globulins. Analysis of disomic and ditelosomic rye/wheat addition lines, following this procedure, has allowed for the assignment of genes encoding LMWP components 1, 3, 5, and 6 to the long arm of chromosome 4R. Additionally, the genes for salt-soluble proteins 7 and 8 have been respectively located in chromosomes 3R and 4R (long arm).

Therefore, genes for the LMWP from rye are located in a different chromosome than those encoding the main prolamins, which have been assigned to chromosomes 1R and 2R (Shepherd and Jennings 1971; Singh and Shepherd 1984; Shewry et al. 1985). A similar situation was found in wheat, where the LMWP genes are in chromosomes 4B, 7A, and 7D (Salcedo et al. 1980) and those corresponding to α, β, γ, and ω-gliadins, LMW- and HMW-glutelins are in chromosome groups 1 and 6 (see Payne et al. 1985).

Previous evidence has indicated that rye chromosome 4R is partly homoeologous to wheat chromosomes of groups 4 and 7 and that the same might be true for chromosome 7R (see Miller 1984). Furthermore, Koller and Zeller (1976) and Zeller and Koller (1981) have proposed a reciprocal translocation event between the ancestral chromosome arms 7RS and 4RL, and have presented evidence of homoeology between rye chromosome arm 4RL and wheat 7BS and 7DS, as well as between rye 7RS and wheat 4AL, 4BS, and 4DL. The chromosomal location of LMWP genes in rye 4RL and wheat 4B, 7AS, and 7DS would point to an ancestral interchange between 7RS and 4RL. Evidence of this translocation has been previously presented by Hart and Langston (1977) and Salinas and Benito (1984) through the analysis of phosphatase isozymes, and by Miller (1984), studying the purple-culm character. Analysis in rye of other biochemical systems associated with wheat chromosome groups 4 and 7, such as the subunits of oligomeric α-amylase inhibitors presently under study in our laboratory, should further clarify this matter.

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References

Aragoncillo C, Sanchez-Monge R, Salcedo G (1981) Two groups of low molecular weight hydrophobic proteins from barley endosperm. J Exp Bot 32:1279–1286

Bidlingmeyer BA, Cohen SA, Tarvin TL (1984) Rapid analysis of amino acids using pre-column derivatization. J Chromatogr 336:93–104

Blakesley RW, Boezi JA (1977) A new staining technique for proteins in polyacrylamide gels using Coomassie Brilliant Blue G-250. Anal Biochem 82:580–582

Charbonnier L, Terce-Laforque T, Mosse J (1981) Rye prolamins: extractability, separation and characterization. J Agric Food Chem 29:968–973

Cottenet M, Feillet P, Autran JC (1984) Isolement et caracterisation de deux gliadines de faible poids moleculaire du ble dur: GFPM 45 et GFPM 42. Comparison avec les γ – gliadines 45 et 42. C R Acad Sci, Ser III 299: 11–16

Drysdale JW, Righetti P, Bunn HF (1971) The separation of human and animal haemoglobins by isoelectric focusing in polyacrylamide gel. Biochim Biophys Acta 229:42–49

Garcia-Olmedo F, Carbonero P, Jones BL (1982) Chromosomal locations of genes that control wheat endosperm protein. In: Advances in cereal science and technology, vol V. AACC, St. Paul, Minn, pp 1–47

Hart GE, Langston PJ (1977) Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. Heredity 39:263–277

Hirs CHW (1967) Performic acid oxidation. Methods Enzymol 11:197–199

Koller OL, Zeller FJ (1976) The homoeologous relationship of rye chromosome 4R and 7R with wheat chromosomes. Genet Res 28:177–188

Kreis M, Forde BG, Rahman S, Miflin BJ, Shewry PR (1985) Molecular evolution of the seed storage proteins of barley, rye and wheat. J Mol Biol 183:499–502

Laemmli VK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 681–685

Miller TE (1984) The homoeologous relationship between the chromosomes of rye and wheat. Current status. Can J Genet Cytol 26:578–589

Moore S, Stein WH (1963) Chromatographic determination of amino acids by the use of automatic recording equipment. Methods Enzymol 6:819–831

Payne PI, Holt LM, Jarvis MG, Jackson EA (1985) Two dimensional fractionation of the endosperm proteins of bread wheat (*Triticum aestivum*): biochemical and genetic studies. Cereal Chem 62:319–326

Paz-Ares J, Hernandez-Lucas C, Salcedo G, Aragoncillo C, Ponz F, Garcia-Olmedo F (1983) The CM-proteins from cereal endosperm: immunochemical relationships. J Exp Bot 34:388–395

Prada J, Sanchez-Monge R, Salcedo G, Aragoncillo C (1982) Isolation of the major low molecular weight gliadins from wheat. Plant Sci Lett 25:281–289

Salcedo G, Rodriguez-Loperena MA, Aragoncillo C (1979) Low MW gliadin-like proteins from wheat endosperm. Phytochemistry 18:725–727

Salcedo G, Prada J, Sanchez-Monge R, Aragoncillo C (1980) Aneuploid analysis of low molecular weight gliadins from wheat. Theor Appl Genet 56:65–69

Salcedo G, Sanchez-Monge R, Argamenteria A, Aragoncillo C (1982) Low molecular weight prolamins: purification of a component from barley endosperm. J Agric Food Chem 30: 1155–1157

Salinas J, Benito C (1984) Phosphatase isozymes in rye. Characterization, genetic control and chromosomal location. Z Pflanzenzücht 93:115–136

Shepherd KW, Jennings AG (1971) Genetic control of rye endosperm protein. Experientia 27:88–98

Shewry PR, Bradberry D, Franklin J, White RP (1985) The chromosomal locations and linkage relationships of the structural genes for the prolamin storage proteins (secalins) of rye. Theor Appl Genet 69:63–69

Shewry PR, Miflin BJ (1985) Seed storage proteins of economically important cereals. In: Advances in cereal science and technology, vol VII. AACC, St. Paul, Minn, pp 1–83

Singh NK, Shepherd KW (1984) Mapping of the genes controlling high-molecular-weight glutelin subunits of rye on the long arm of chromosome 1R. Genet Res 44: 117–123

Zeller FJ, Koller OL (1981) Identification of a 4A/7R and a 7B/4R wheat-rye chromosome translocation. Theor Appl Genet 59:33–37