Chromosomal assignment of genes controlling salt-soluble proteins (albumins and globulins) in wheat and related species

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Summary. Salt-soluble proteins from the endosperms of wheat, barley, and rye have been separated by non-equilibrium electrofocusing × electrophoresis. Genes encoding 14 of the 25 components observed in wheat have been unambiguously assigned to 10 different chromosomes (1B, 3B, 3D, 4A, 4D, 5B, 6B, 6D, 7B, 7D) by analysis of the compensated nulli-tetrasomic series. Five more wheat proteins seem to be controlled by group 2 chromosomes. Analysis of wheat-barley and wheat-rye addition lines has led to the location of genes for 6 out of 20 barley proteins in 4 different chromosomes (1H, 3H, 4H, 6H; 1H is homoeologous to group 7 chromosomes of wheat) and of genes for 5 out of 20 rye proteins in two different chromosomes (2R, 4R). The relationship between the proteins reported here and previously characterized ones is discussed.

Key words: Chromosomal location — Genetic control — Salt soluble proteins — Wheat — Barley — Rye

Introduction

The cereal endosperm is one of the main sources of dietary protein for man and domestic animals. The nutritional value and certain technological properties of the cereal grains largely depend on the protein content and composition of this tissue. Numerous studies have been carried out on the biochemistry and genetics of a wide variety of endosperm protein components in the different cereal species. Most of these studies have dealt with the prolamins, storage proteins that normally represent more than half of the kernel protein and are deficient in certain essential amino acids, especially lysine. The soluble proteins, albumins and globulins, which may represent up to one fourth of the kernel protein and close to one half of the total lysine, have received less attention, in spite of their obvious nutritional relevance. In the case of wheat, barley, and rye, the genetic control of different protein components has been extensively studied using the diverse spectrum of aneuploid strains originally obtained by Sears (1954, 1966) and further extended in many laboratories around the world (Riley and Chapman 1958; Morris et al. 1966; Islam et al. 1975; and others). Although in some of these studies, the genetic control of specific groups of albumins and globulins has been investigated (for a review, see Garcia-Olmedo et al. 1982), no systematic study of the chromosomal locations of genes encoding these proteins has been carried out using high-resolution two-dimensional methods. We report here such a study for albumins and globulins from wheat, barley, and rye.

Materials and methods

Biological materials

The complete series of compensated nulli-tetrasomics from *Triticum aestivum* cv. 'Chinese Spring' (except those nulli-somic for chromosomes 2A and 4A) and ditelosomic 4Aα were the gift of E. R. Sears from the University of Missouri (Sears 1954, 1966). Disomic addition lines of chromosomes 1, 2, 3, 4, 6 and 7 from *Hordeum vulgare* cv. 'Betzes' in a *T. aestivum* cv. 'Chinese Spring' background (Islam et al. 1978) were donated by Dr. K. W. Shepherd (Adelaide, Australia). The complete set of disomic addition lines, carrying chromosomes of *Secale cereale* cv. 'Imperial' in a background of *T. aestivum* cv. 'Chinese Spring' were obtained from Dr. E. R. Sears.

Extraction and fractionation of proteins

Individual kernels (40−50 mg) were placed between two metal plates and crushed with a hammer. Lipids were extracted in a
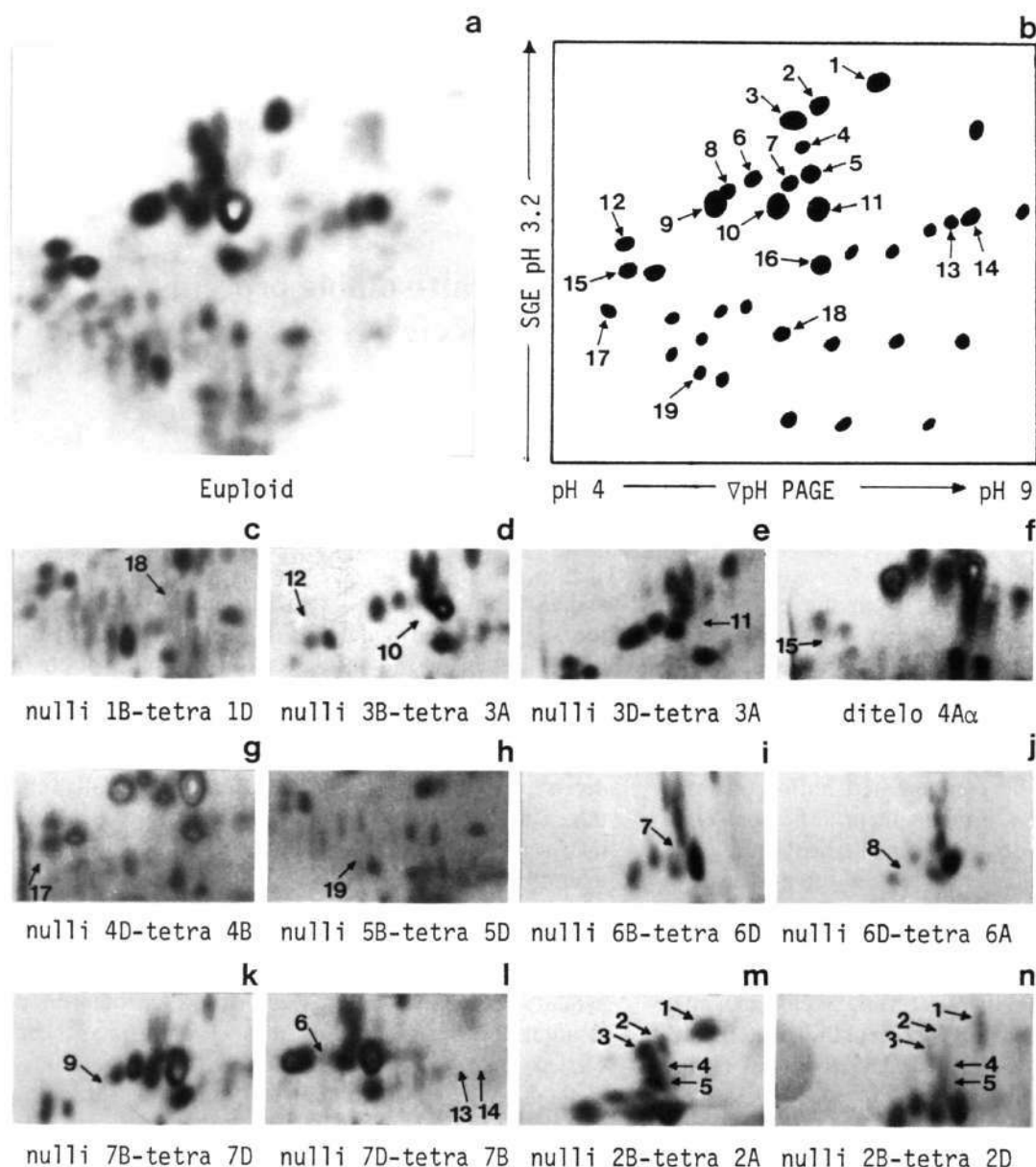
Fig. 1a–n. Aneuploid analysis of albumins and globulins of wheat. **a** Fractionation of combined non-equilibrium electrofocusing (1st dimension) × electrophoresis (2nd dimension) of salt-extracted proteins from euploid *T. aestivum* cv. 'Chinese Spring'. **b** Schematic representation of the two-dimensional protein map shown in Fig. 1a. Components alluded to in the text have been numbered. **c–n** Pertinent zones of the two-dimensional maps corresponding to the indicated aneuploids. Arrows point to the positions of the missing proteins, which in each case are identified by their corresponding numbers

small tube with 10 volumes of petroleum ether (bp 40–70 °C) at room temperature, and the residual flours were air-dried. Albumins and globulins were extracted from the flours with 0.5 M NaCl at 4 °C (2 × 10 v/w; 1 h). After centrifugation of the extraction mixtures at 6,000 × g for 15 min, proteins were precipitated from the supernatants with trichloroacetic acid (12.5%, final concentration). The precipitates were washed with 400 µl of acetone, air-dried, and stored at −20 °C until used. The dried samples were dissolved in 50 µl of 9 M urea and fractionated by two-dimensional electrophoresis: first dimension, non-equilibrium electrofocusing on preformed pH gradients (pH 4–9; 5% polyacrylamide; 2 × 140 mm column; 67 V cm^{-1}, 2–3 h); second dimension, starch-gel electro-

phoresis (0.1 M aluminium lactate buffer, pH 3.2, 3 M urea; 1 mm × 18 cm × 28 cm slabs; 20 V cm^{-1}, 4 h). The pH gradients (ampholines pH 4–9; buffers 0.2% H$_2$SO$_4$ and 0.4% ethanol-amine) were prefocused at 0.5 mA per tube until 470 V were reached (∼ 40 min). Gels were stained with 0.05% nigrosine in methanol-water-acetic acid (5 : 5 : 1, by vol) for 16 h.

Results

The two-dimensional fractionation of the salt extract from euploid 'Chinese Spring' wheat is shown in Fig. 1a. Preliminary experiments were carried out, at dif-
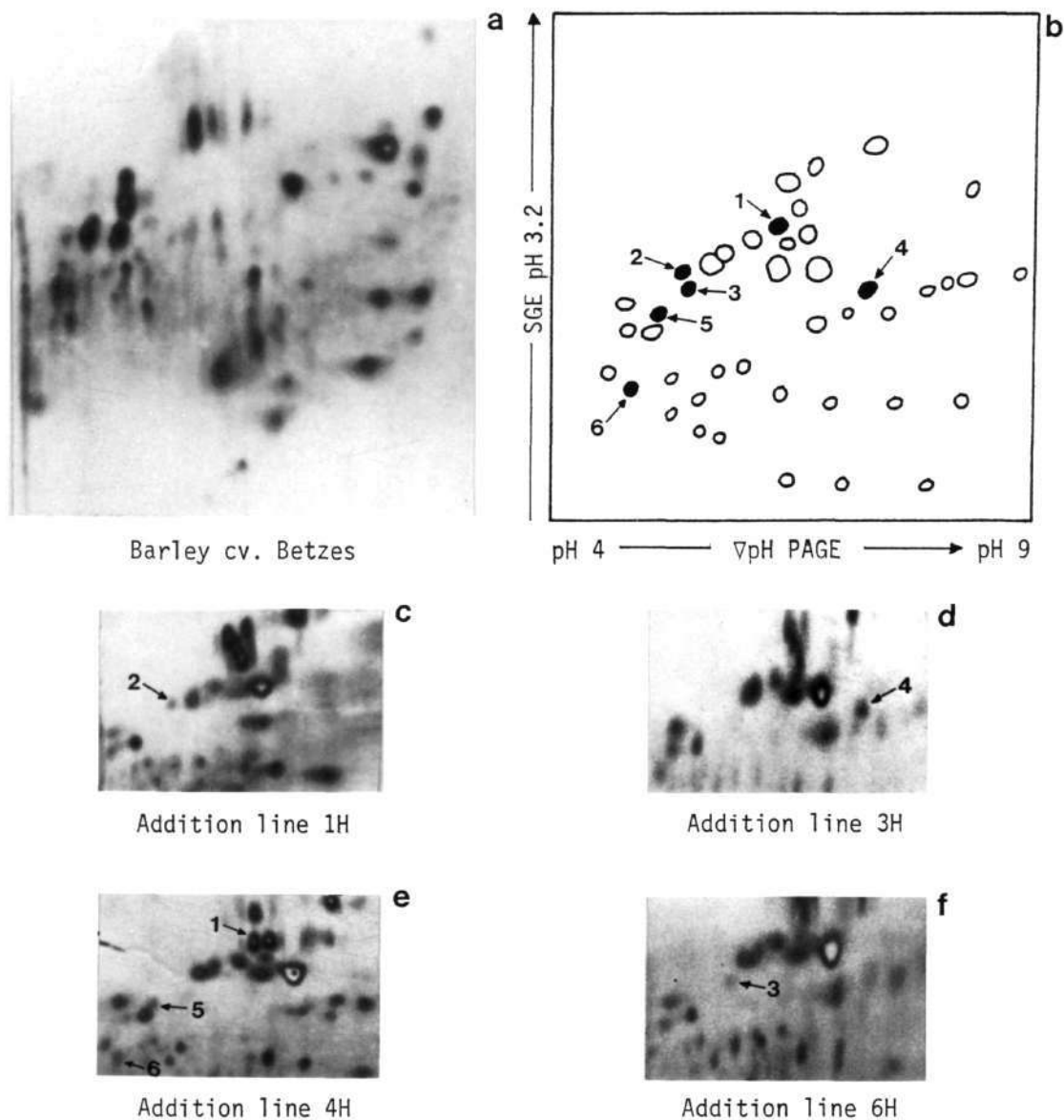
Fig. 2a–f. Aneuploid analysis of albumins and globulins of barley. **a** Fractionation as in Fig. 1 of salt-extracted proteins from *H. vulgare* cv. 'Betzes'. **b** Schematic representation of the barley proteins *(black spots)* that can be unequivocally identified in the background of wheat proteins *(open spots* corresponding to those represented in Fig. 1b). **c–f** Pertinent zones of the two-dimensional maps corresponding to the indicated 'Chinese Spring'/'Betzes' wheat/barley addition lines. *Arrows* and *numbers* indicate the additional proteins appearing in each of the addition lines shown

ferent times of non-equilibrium electrofocusing in the first dimension, to ascertain that all major components of this protein fraction (albumins and globulins) were included in the map, and the final conditions were chosen so that optimum resolution was obtained. No precipitate was observed in the sample solution during electrofocusing. About 25 main components were reproducibly observed by this procedure. No differences were observed for the main components between hand dissected endosperms and crushed whole kernels. The chromosomal assignment of genes encoding these pro-

teins has been carried out by the two-dimensional analysis of compensated nulli-tetrasomic stocks and of ditelosomic 4Aα. Proteins for which information was obtained in the analysis have been numbered in the schematic representation of Fig. 1b. Genes encoding 13 of the main proteins (components 6–15, 17–19) were unequivocally assigned to ten different chromosomes (Table 1) on the basis of the absence of each protein in all strains lacking a particular chromosome. Although the expected dosage effects were observed in most cases, no attempt was made at densitometric
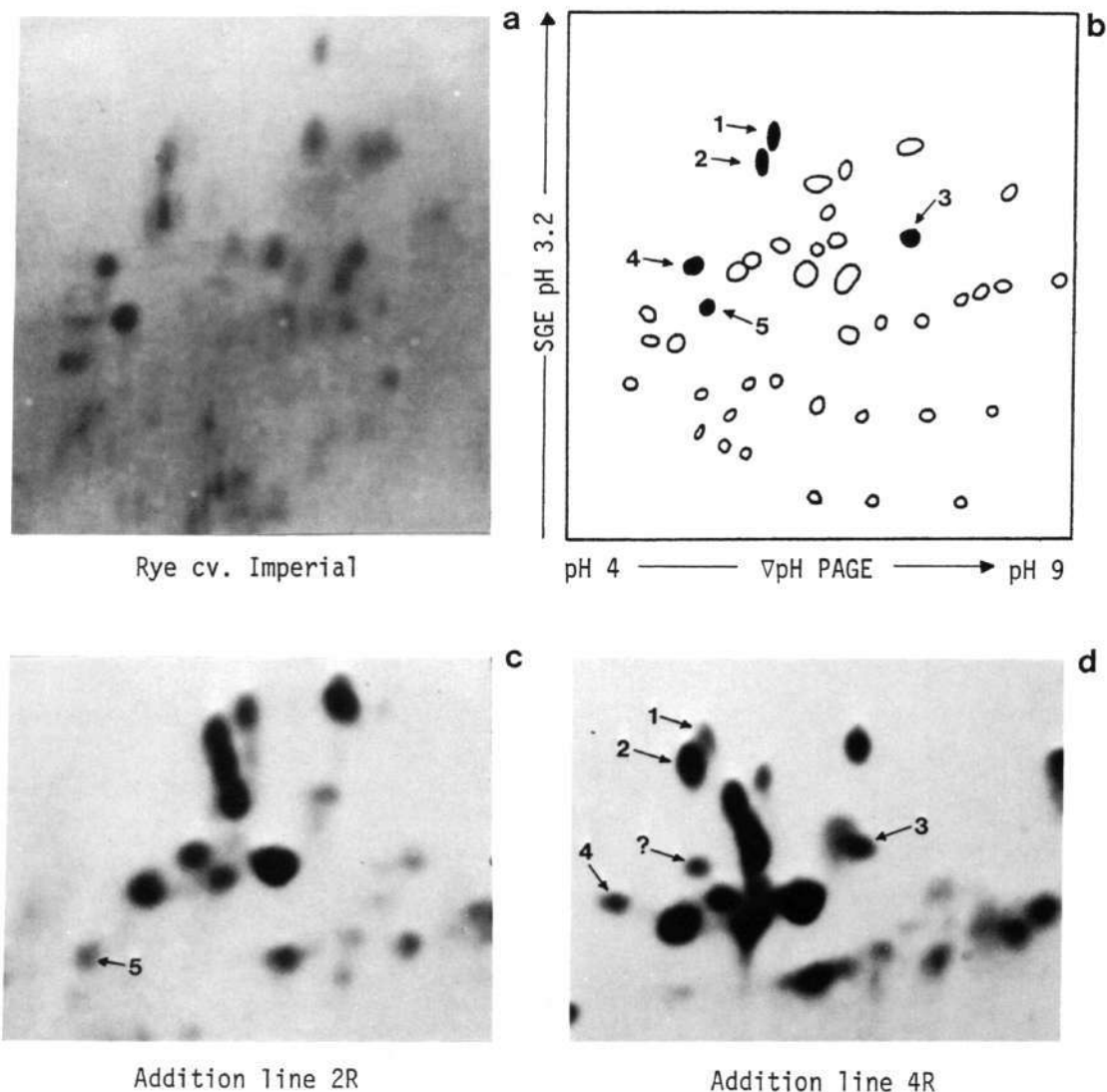
Rye cv. Imperial

a
SGE pH 3.2
pH 4 ———— ∇pH PAGE ————→ pH 9
b

Addition line 2R

Addition line 4R

Fig. 3a–d. Aneuploid analysis of albumins and globulins of rye. **a** Fractionation as in Fig. 1 of salt-extracted proteins from *S. cereale* cv. Imperial. **b** Schematic representation of the rye proteins *(black spots)* that can be unequivocally identified in the background of wheat proteins *(open spots* corresponding to those represented in Fig. 1b). **c–d** Pertinent zones of the two-dimensional maps corresponding to the indicated 'Chinese Spring'/'Imperial' wheat/rye addition lines. *Arrows* and *numbers* indicate the additional proteins appearing in each of the addition lines shown. The *arrow* identified with a question mark in Fig. 3d points to a component that is not detected in either wheat or rye

quantitation of the spots. Relevant parts of the two-dimensional maps showing the absence of the different proteins in appropriate aneuploid strains are presented in Fig. 1c–l. Component 16 corresponds to the previously studied protein CM3, which was found to be encoded by at least two genes, located in chromosome 4A and in an unknown chromosome of the D genome (Rodriguez-Loperena et al. 1975; Aragoncillo et al. 1975). Five additional proteins (components 1–5) were found at very low levels in nulli 2B-tetra 2D and at euploid levels in nulli 2B-tetra 2A (Fig. 1m–n).

The separation of barley albumins and globulins by two-dimensional electrophoresis is shown in Fig. 2a.

About 20 main components were detected. Considerable overlap between barley and wheat components was observed when a mixture of the salt extracts from the two species was subjected to joint electrophoresis. Fig. 2b is a diagram in which the barley proteins not overshadowed by wheat ones, and whose genes we were able to assign, are numbered and shown as black spots. Genes for these proteins were assigned to four different chromosomes by analysis of the six available wheat-barley addition lines (Fig. 2c–f; Table 1; addition 5 not available). It is to be noted that, in the disomic addition of chromosome 4H of barley (Fig. 2e), the expression of the barley genes corresponding

Table 1. Chromosomal assignment of genes encoding salt-soluble proteins in wheat and related species

Extractant	Genomes[a]	1	2	3	4	5	6	7	References[b]
0.5 M NaCl	A		5?		2				1
	B	1		2		1	1	1	
	D			1	1		1	3	
	H			1	3		1	1	
	R		1		4				
70% ethanol	A				2				2, 3
(MW < 25,000)	B			2			2	1	
	D			1	1	1		2	
$Cl_3CH/MeOH$	A				2				2–4
(2/1; v/v)	B							1	
	D				1			2	
	H			1	2			2	5

[a] T. aestivum (genomes ABD); H. vulgare (H); S. cereale (R). Chromosomes of the H genome have been numbered according to their homoeology with wheat chromosomes

[b] 1) Present report, 2) Aragoncillo et al. 1975, 3) Rodriguez-Loperena et al. 1975, 4) Garcia-Olmedo and Carbonero 1970, 5) Salcedo et al. 1984

to proteins 1, 5 and 6, was concomitant with the lack of expression of the genes encoding wheat components 2 and 4 from Fig. 1 b.

Chromosomal locations of genes encoding albumins and globulins in rye were investigated in a similar manner as those in barley. About 20 main components were detected (Fig. 3a). Wheat-rye disomic addition lines were analysed by the same two-dimensional method and genes encoding 5 proteins were assigned to two chromosomes (Fig. 3b–d; Table 1). Two additional map components appeared in addition lines 1 R and 4 R that could not be detected in either T. aestivum cv. 'Chinese Spring' or in S. cereale cv. 'Imperial' or in mixtures of the two samples.

Discussion

The salt extracts of wheat, barley and rye, which represent 15—25% of the total endosperm protein, consist of 20—25 individual components, as shown by the electrophoretic procedure used in the present study. From these data, it may be concluded that, on the average, each of these proteins represents about 0.5—1.0% of the total protein. This indirect estimate has been confirmed for some proteins by a more direct densitometric procedure using the purified proteins as standards (unpublished). This means that many albumins and globulins are as abundant in the endosperm

as many individual components of the typical reserve prolamins (50—60% of the total endosperm protein; up to 50 components in wheat). These data and the fact that the salt-soluble proteins are also used, together with the prolamins, as substrates by the germinating embryos, tend to obscure the traditional assumption of an exclusively non-storage function for albumins and globulins.

Based on the chromosomal locations of their genes and on their positions in the two-dimensional maps, 9 out of the 14 salt-soluble wheat proteins unambiguously assigned in the present work are identical with components of the 70% ethanol extract (Table 1) previously reported by Aragoncillo et al. (1975). Components 18 (chromosome 1B), 19 (chromosome 5B), 8 (chromosome 6B) and 13 and 14 (chromosome 7D) correspond to new assignments. The observations concerning components 1–5, which are not included in the 70% ethanol extracts, could be explained by the existence of duplicate sets of genes for these proteins, respectively located in chromosomes 2A and 2B or, alternatively, by some sort of regulatory or modifier effect.

In this context, it should be pointed out that several laboratories have reported effects of this type on certain wheat prolamins by group 2 chromosomes (Shepherd 1968; Waines 1973; Brown and Flavell 1981). About one fourth of the components of the wheat two-dimensional map did not show variation among the aneuploid strains investigated and therefore no indication about the chromosomal locations of the corresponding genes could be obtained. This could be due either to the location of these genes in the unexplored part of the genome (chromosome 2A; α arm of chromosome 4A; or cytoplasmic genomes) or to the existence of triplicate homoeologous genes encoding each of these map components.

Four of the six components assigned in barley correspond to previously reported components of the chloroform : methanol extract (Salcedo et al. 1984). Component 1 (chromosome 4H) and 3 (chromosome 6H) had not been previously assigned. Components 2, 4, 5 and 6 are identical with proteins CMa-1, CMe-1, CMb-1 and CMd-1, respectively (Salcedo et al. 1984). However, we fail to detect, under the present analytical conditions, component CMc-1 encoded by chromosome 1H (equivalent to wheat homoeology group 7) in spite of the fact that it is at least partially salt-soluble (Salcedo et al. 1982, 1984). To our knowledge, no genes encoding salt-soluble proteins had been previously located in rye. A greater proportion of genes encoding albumins and globulins from barley and rye have remained unassigned because of the overlap of the proteins of these species with those of wheat in the addition lines. The lack of expression of some wheat genes in one barley addition line (4H disomic addition) and, in the case of wheat-rye addition lines, the appearance of components that were not detected in the cultivars from which the lines were derived (1 R

and 4R disomic additions), seem to indicate that both negative and positive genetic interactions can take place between the added chromosomes and those of the recipient species. However, heterogeneity in the initial parental material can not be excluded.

Most of the salt-soluble proteins encoded by genes located in chromosomes of groups 4 and 7 in wheat and chromosomes 4 and 1 of barley are soluble in chloroform: methanol mixtures and have been designated CM-proteins (Aragoncillo et al. 1975; Salcedo et al. 1984). We have presented biochemical and immunochemical evidence of sequence homology among CM-proteins encoded by genes located in both groups of chromosomes in the two species (Salcedo et al. 1982; Paz-Ares et al. 1983). In the case of rye, we have only preliminary evidence that at least two of the proteins assigned to chromosome 4 are CM-proteins, and have not yet been able to ascertain whether or not there are also genes for salt-soluble proteins in chromosome 7. An answer to this question could contribute to further clarify the proposed relationship of chromosome 4R of *S. cereale* with homoeologous groups 4 and 7 of wheat (Koller and Zeller 1976). The possibility that at least some of the proteins encoded by genes located in chromosomes of group 3 might be structurally related to the CM-proteins has been recently raised (Salcedo et al. 1984, and unpublished results) and is being actively investigated. This would imply that at least some of the main components of the salt-soluble protein fraction are members of a widespread multigene family.

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