Chromosomal location and expression of genes encoding low molecular weight proteins in wheat and related species

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Summary

A review of recent work on the chromosomal location and expression of genes encoding moderately abundant, presumably non-storage endosperm proteins from wheat, barley and rye is presented. Related aspects, such as regulatory genetic effects, *in vivo* and *in vitro* synthesis, types of processing, deposition sites, and molecular cloning are also discussed. The relevance of these studies in connection with basic endosperm biology, genetic manipulation of quality and agronomic traits, and the evolution of these important crops is briefly emphasized.

Introduction

The purpose of this paper is to review some recent findings, together with previously published work, concerning the genetics and molecular biology of different types of low molecular weight proteins, which are moderately abundant in the cereal endosperm, but are not typical storage proteins by a number of criteria. The study of these proteins is of interest in connection with the achievement of a more complete understanding of endosperm biology, which will be required in future genetic manipulations of the composition of this tissue. Knowledge of the chromosomal location of genes encoding these proteins has also allowed their use as biochemical chromosome markers in the interspecific transfer of genes controlling agronomic traits, such as resistance to certain diseases (see Rodriguez-Loperena et al. 1975b; Hart et al. 1976; Delibes et al. 1981; Doussinault et al. 1983). Certain of these proteins have been of diagnostic value in relation to the study of the origin and evolution of cereal species (Johnson and Hall 1965; Hall et al. 1966; Johnson 1972, 1975; Salcedo et al. 1984). The general features of endosperm protein composition have been found to be quite similar in many of the cereal species investigated. Therefore, this review will not be restricted to wheat, but will integrate information concerning other Gramineae, specially barley and rye.

Types of proteins investigated

The albumin and globulin fractions of cereals are made up of over 20 major and many minor components, most of which are under 25,000 molecular weight and are included in the main peak of a typical gel filtration profile (CALDWELL and KASARDA 1978; SALCEDO et al. 1980b; RAHMAN et al. 1982).

The complexity of these fractions has made difficult to study the genetic control of many of their individual components by one-dimensional electrophoresis or electrofocusing. This has led to the development of different procedures to partially purify or selectively extract subsets of proteins and to the application of a variety of two-dimensional separation methods (Fig. 1). Thus, a number of components of the albumin and globulin fractions are extracted by aqueous alcohols, and a subset of those soluble in aqueous alcohols, designated CM-proteins, are soluble in chloroform: methanol (2 : 1, v/v) (GARCIA-OLMEDO and GARCIA-FAURE 1969; RODRIGUEZ-LOPERENA et al. 1975a). The CM-proteins also appear residually in prolamin preparations, where they are among the most abundant low molecular weight components (see SALCEDO et al. 1980b). This information is summarized in Fig. 2.

Besides the CM-proteins, the fraction under 25,000 molecular weight in prolamin preparations also includes another group of prolamin-like proteins, designated low-molecular-weight prolamins (LMWG in wheat, LMWH in barley), which are insoluble in H_20 and in 0.5 M NaCl (SALCEDO et al. 1979; 1982; ARAGONCILLO et al. 1981; PRADA et al. 1982).

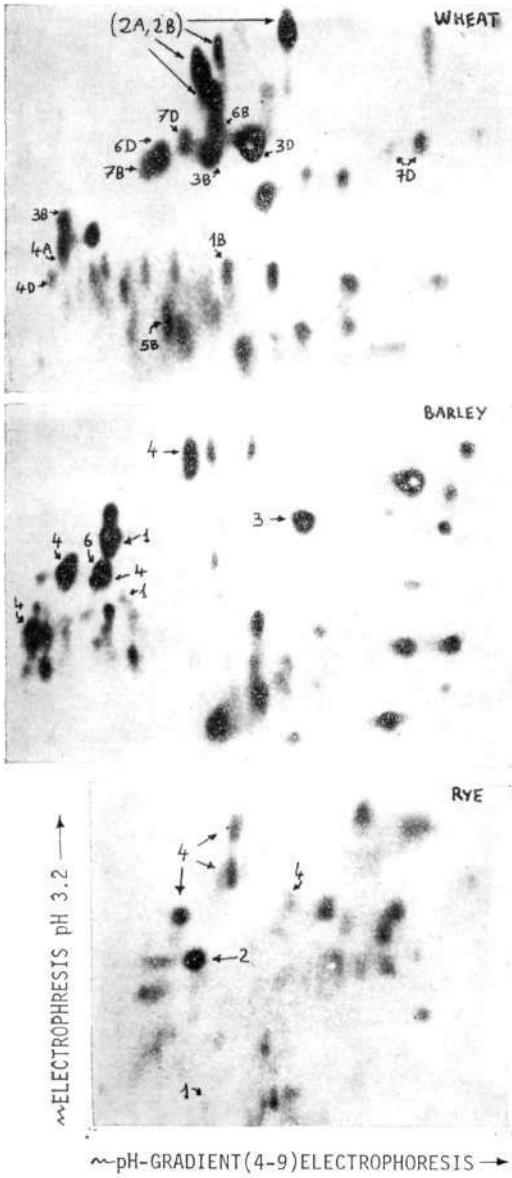

Fig. 1 Two-dimensional fractionation by combined non-equilibrium pH-gradient (4–9) electrophoresis × electrophoresis pH 3.2 of proteins extracted by 0.5 M NaCl from the endosperms of wheat, barley, and rye. Chromosomes where the genes encoding different components of the two-dimensional map are indicated in the figures (FRA-MON et. al. 1984)

Petroleum ether extracts of cereal endosperms include two types of proteins, the thionins (see GARCIA-OLMEDO et al. 1982) and the lipid binding proteins (LBP), both of which are also extracted with 0.5 M NaCl (PONZ et al. 1984).

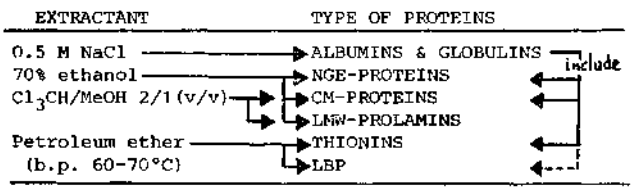

Fig. 2 Relationships among the types of proteins extracted with different solvents from the endosperms of wheat, barley, and rye

Genetic variability and evolutionary implications

In contrast with the considerable intraspecific variability of the main storage prolamins (DOEKES 1968; DOLL and BROWN 1979; SHEWRY et al. 1979), most of the types of proteins described above show little intraspecific and even interspecific variability. For this reason, these proteins have been useful in the elucidation of genome relationships and the analysis of phylogenetic affinities in the Triticineae.

The electrophoretic analysis of the low molecular weight components of the 70 % ethanol extracts of kernel proteins was used by JOHNSON and coworkers (JOHNSON and HALL 1965; HALL et al. 1966; JOHNSON, 1972, 1975) in their extensive studies on the origin of the alloploid Triticineae.

The CM-proteins, which are included in the previous group, have been found to be quite invariant in wheat and barley. In bread-wheat (*Triticum aestivum* L.), protein CM 1 was found to be invariant, while it was absent in tetraploid wheat cultivars (*T. durum* Desf.), which are the adequate ingredient for pasta products, and this protein was used for the detection of the fraudulent use of hexaploid wheat to elaborate these products (GARCIA-OLMEDO and GARCIA-FAURE 1969). Proteins CM 2 and CM 3 have been found to be invariant among tetraploid and hexaploid wheat cultivars, except for two closely related tetraploid ones that presented an allelic variant of CM 3, designated CM 3' (GARCIA-OLMEDO and GARCIA-FAURE 1969; RODRIGUEZ-LOPERENA et al. 1975a; SALCEDO et al.

Table 1
Variability of CM-proteins in *Hordeum vulgare* and *H. spontaneum*

Variant Protein	N° of Samples	
	H. vulgare	*H. spontaneum*
CMa-1	38	13
CMa-2	0	4
CMb-1	30	15
CMb-2	7	1
CMb-3	1	1
CMc-1	38	16
CMc-2	0	1
CMd-1	38	17
CMe-1	21	8
CMe-2, 2'	17	7
CMe-3	0	2

1978). In a recent study (SALCEDO et al. 1984),[1] proteins CMa-1, CMc-1, and CMd-1 were found to be invariant among *Hordeum vulgare* cultivars, the latter being invariant among *Hordeum spontaneum* accessions (Fig. 3 and Table 1). Certain variants of CMb-1 and CMe-1 seem to be restricted to *H. vulgare* and *H. spontaneum* samples from Morocco and to cultivars with possible Moroccan origin. This has led to speculate that a domestication event possibly occurred in that area (MOLINA-CANO et al. in preparation).

In a survey of *Aegilops-Triticum* species, CARBONERO and GARCIA-OLMEDO (1969) observed little variability and a certain genome specificity for the thionins. Subsequent sequence studies have confirmed these findings (for a review, see GARCIA-OLMEDO et al. 1982). The amino acid sequence of β-thionin has not changed in the evolution from diploid to hexaploid wheat and, in fact, there is considerable homology between thionins in cereals and similar proteins in very distant *taxa*, such as the viscotoxins of mistletoe (*Viscum album*) and crambin from *Crambe abyssinica*.

Considerable variability was observed for the low molecular weight gliadins among tetraploid and hexaploid wheat cultivars, although they were not as variable as the classical gliadins (SALCEDO et al. 1980a).

The variability of LBPs has not been investigated, but PONZ et al. (1984) have found close homology between the LBPs of wheat and oats.

Chromosomal locations of genes encoding low molecular weight proteins in wheat and related species

A considerable number of genes encoding proteins alluded in this review have been assigned to their corresponding chromosomes by aneuploid genetic analysis. A summary of previously published information is given in Table 2 (for a detailed review, see GARCIA-OLMEDO et al. 1982). More recent work along this line has dealt with the chromosomal location of genes encoding salt-soluble proteins in wheat, rye and barley (FRA-MON et al., 1984) and of genes encoding CM-proteins in barley (SALCEDO et al. 1984).

The 0.5 M NaCl extracts of the nulli-tetrasomic series of Chinese Spring wheat, the Imperial rye/Chinese Spring wheat addition lines, and the Betzes barley/Chinese Spring addition lines were analyzed by two-dimensional pH gradient (4–9) polyacrylamide gel electrophoresis × starch-gel electrophoresis (pH 3.2) as indicated in Fig. 1. Genes for 17 of the most prominent components of the wheat two-dimensional map have been assigned to 12 different chromosomes. In 9 of the cases (2 proteins associated with chromosome 3B, 1 with 3D, 1 with 4A, 1 with 4D, 1 with 6B, 1 with 6D, 1 with 7B, 1 with 7D), the genes had been previously located using more selective protein extraction procedures (GARCIA-OLMEDO and CARBONERO 1970; ARAGONCILLO et al. 1975). The remaining four cases (1 protein in 1B, 1 in 5B, 2 in 7D) correspond to new assignments. Additionally, it has been observed, that group 2 chromosomes affect the expression level of 4 more proteins. Genes encoding a number of minor components have been also tentatively located.

Due to the complexity of the background of wheat proteins, a smaller number of map components of rye and barley, have been assigned through the analysis of

Table 2

Summary of chromosomal locations of genes for non-storage endosperm proteins from wheat and related species

Type of protein+ (Separation method)	References++	Chromosome homoeology group N°* 1	2	3	4	5	6	7
Thionins (E)	a	AL (1) BL (1) DL (1) RL (1)						
70 % ethanol (E) (low mol. wt.)	b				A (2?)			B (1)
				D (1)	D (1?)			D (1)
(E×IEF)	c				Aβ (1+1?)			
				BS (2)			BS (2)	BS (1)
				DS (1)	D (1)	D (1)		DS (2)
				AgS (1)				AgS (2)
				R (1)				
CM-proteins (E×IEF)	c				Aβ (2)			
								BS (1)
					D (1)			DS (2)
								AgS (2)
	d			H (1)	H (2)			H (2)
	e				R (2?)			
Globulins (E)	f			A (1)				
				B (2)				
				D (4)				
Albumins (imm)	g			D (1)	D (1)			
Buffer sol. (IEF)	h	B (1?)		AS (1?)	A (2?)		A (2?)	
				BS (1+1?)				
				DS (1)				
0.5 M NaCl (E×E)	e		A } (4?)		A (1)			
		B (1)	B }	B (2)		B (1)	B (1)	B (1)
				D (1)	D (1)		D (1)	D (3)
				H (1)	H (4)			H (2)
		R (1)	R (1)		R (4)			
LMWG (E×E)	i							AS (2)
					B (2)			
								DL (2)

\+ Type of protein is indicated by common designation or by extractant used; method of separation is indicated in parenthesis, E = one-dimensional electrophoresis, IEF = isoelectrofocusing, E×E = two-dimensional electrophoresis, imm = immunochemical analysis

++a) Garcia-Olmedo et al., 1976; Fernandez de Caleya et al., 1976; Sanchez-Monge et al., 1979. b) Waines, 1973. c) Garcia-Olmedo and Carbonero, 1970; Aragoncillo et al., 1975; Rodriguez-Loperena et al., 1975. d) Salcedo et al., 1984. e) FraMon et al., 1984. f) Cubadda, 1975. g) Bozzini et al., 1971. h) Noda and Tsunewaki, 1972. i) Salcedo et al., 1980.

* The genome is indicated first (ABD, wheat; Ag, Agropyron; H, Hordeum; R, rye), followed by the chromosome arm (L, long; S, short; α or β). The number of components assigned is indicated in parenthesis, followed by the question mark when there is overlapping of bands. Chromosome 1 of barley has been placed under homeology group n° 7.

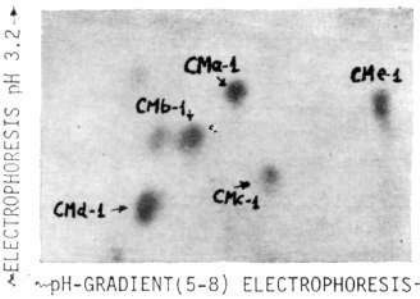

Fig. 3 Two-dimensional fractionation of CM-proteins from barley *(Hordeum vulgare)*

addition lines. In the case of rye, one map component was associated with chromosome 1, one with chromosome 2, and four with chromosome 4. In the case of barley, genes for eight components were located in four chromosomes: two in chromosome 1, one in chromosome 3, four in chromosome 4, and one in chromosome 6.

Among the four salt-soluble proteins associated with chromosome 4 of rye, two of them, and possibly a third one, have been tentatively identified as CM-proteins. In the case of barley, the chromosomal location of genes encoding CM-proteins has been directly investigated in the wheat-barley addition lines (SALCEDO et al., 1984). Genes encoding proteins CMa and CMc were located in chromosome 1, genes for proteins CMb and CMd were assigned to chromosome 4, and the newly reported protein CMe was associated with chromosome 3 (Fig. 3). Sequence homology, among proteins CMa, CMb, CMc, and CMd purified from barley and between the barley and the wheat CM-proteins has been inferred on the basis of compositional divergence indexes and of immunological evidence (SALCEDO et al. 1982; PAZ – ARES et al. 1983a). In particular, CM 3 from wheat and CMd from barley showed the closest interspecific relationship: complete antigenic identity, the only CM-proteins in each species to be extracted with the 7 : 1 (v/v) mixture of chloroform : methanol, and the lowest compositional divergence index. Genes for the chloroform : methanol soluble proteins of wheat have been ascribed to chromosomes of groups 4 and 7, and, in particular, the gene for protein CM 3 was found to be located in chromosome 4A (GARCIA-OLMEDO and CARBONERO 1970; ARAGONCILLO et al. 1975). The present assignment of genes for the four barley proteins to chromosomes 1 and 4, further supports the previously proposed homoeology between chromosome 1 of barley and group 7 of wheat and between chromosome 4 of barley and group 4 of wheat (HART et al. 1980; POWLING et al. 1981).

Regulatory effects

A quantitative study of gene-dosage responses was conducted by ARAGONCILLO et al. (1978) with a group of six low molecular weight, 70 % ethanol soluble proteins from wheat endosperm, which were encoded by incomplete (not triplicate) homoeologous gene sets. Approximately linear dosage responses were observed for all the proteins. For two of the proteins, and probably for a third one, the net output of protein for each dose of its structural gene was 30–80 % higher when the chromosome carrying an active homoeogene was absent. These observations

indicated that gene-dosage responses for some endosperm proteins can be modified by genetic elements located in a chromosome different from that containing their structural genes. In a related study, SALCEDO et al. (1978) showed differences in gene-dosage responses among alleles at a locus encoding proteins CM 3 and CM 3' in *T. turgidum*. The net number of protein molecules present in the mature endosperm was measured when each of the alleles was present in one, two, and three doses. Linear gene-dosage responses were again observed, but for a given dosage, about twice as much CM 3 as CM 3' protein was found. Genetic evidence indicated that the observed quantitative differences either resulted from differences, in the structural genes themselves or were controlled by regulatory or modifier gene(s) linked to them.

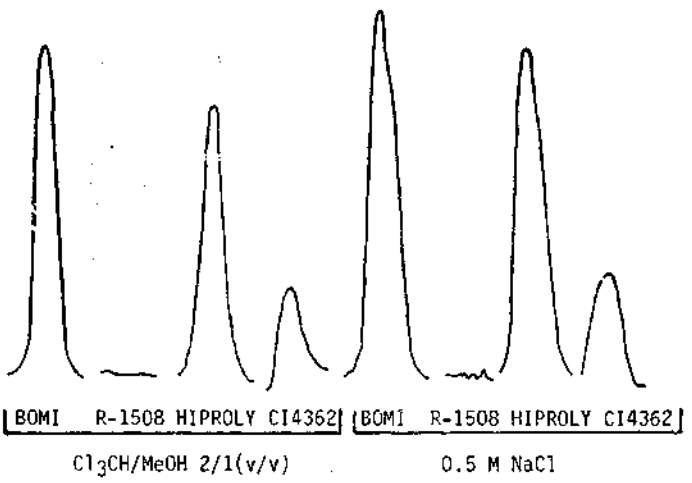

Fig. 4 Densitometric evaluation of protein CMe-1 extracted with chloroform/methanol 2/1 (v/v) or with 0.5 M NaCl after two-dimensional fractionation. Bomi barley and its mutant Ris 1508, HIPROLY barley and its sister line CI4362 were compared in the same gel slab. In the legend of the curves HIPROLY and C 14362 must be exchanged.

In our recent study of the genetic variability and control of CM-proteins in barley (SALCEDO et al. 1984), a different type of genetic effect has been shown to occur: expression of gene(s) encoding protein CMe, located in chromosome 3, is completely blocked by the "high lysine" mutation in Riso 1508, which locus is in chromosome 7. The accumulation of this protein is markedly decreased in the "high lysine" barley Hiproly (Fig. 4). It has been shown that B and C-hordeins are drastically decreased in the Ris 1508 mutant and, to a lesser extent, in Hiproly barley, while the salt-soluble fraction is increased in both mutants (see MIFLIN and SHEWRY 1979). RHODES and GILL (1980) reported that a salt-soluble component was decreased in Ris 1508 and HEJGAARD (1982) found that protein Z was decreased in Ris 1508, but greatly increased in Hiproly. To our knowledge, CMe is the only salt-soluble protein whose accumulation is totally or partially blocked in both Ris 1508 and Hiproly.

Synthesis, processing and deposition of proteins in endosperm

Synthesis, processing and deposition of proteins in the cereal endosperm have been intensively studied in recent times. Storage proteins from maize (BURR et al. 1978; LARKINS and HURKMAN 1978; WIENAND and FEIX 1978; VIOTTI et al. 1979),

barley (BRANDT and INGVERSEN 1978; MATTHEWS and MIFLIN 1980), wheat (GREENE 1981; OKITA and GREENE 1982; DONOVAN et al. 1982), rice (YAMAGATA et al. 1982) and oats (LUTHE and PETERSON 1975; MATLASHEWSKI et al. 1982) have atracted most of the interest. In this general context, we have focused our attention on the thionins and CM-proteins of wheat, barley and rye. The initial *in vivo* and *in vitro* studies have been carried out in developing barley endosperm (PAZ-ARES et al. 1983b; PONZ et al. 1983) and some of the observations have been also extended to wheat (unpublished).

PAZ-ARES et al. (1983b) have reported that CM-proteins in barley are synthesized non-synchronously from 10 to 30 d after anthesis by membrane-bound polysomes as precursors of higher apparent molecular weight (13,000–21,000) than the mature proteins (12,000–16,000). These precursors are processed and the mature protein exported into the cytosol. PONZ et al. (1983) detected thionin synthesis from ~8 d to ~30 d after anthesis. They identified two thionin precursors (THP 1 and THP 2) using monospecific antibodies raised against the mature protein. THP 1 is the only polypeptide among the *in vitro* products that is recognized by the monospecific antibodies. THP 1 is encoded by a 7.5 S mRNA and its alkylated derivative has an apparent molecular weight of 17,800. THP 2, which is selected together with mature thionin by the antibodies among labelled proteins *in vivo*, differs from THP 1 in apparent molecular weight (17,400 alkylated) and in electrophoretic mobility at pH 3.2. Both THP 1 and THP 2 were competed out from the antigen-antibody complex by purified thionin. Final deposition of the mature protein takes place in the particulate fraction as an extrinsic protein.

Pulse-chase *in vivo* experiments showed that the conversion of THP 2 into thionin was a post-translational event, whereas the presumed conversion of THP 1 into THP 2 was assumed to be co-translational on the basis of our failure to detect THP 1 *in vivo*. The processing of the precursors of CM-proteins was also assumed to be co-translational by the same criterium. We have now obtained additional evidence in support of these tentative conclusions through *in vitro* translation experiments with initiation-inhibited bound polysomes and with the dog-pancreas *in vitro* processing system.

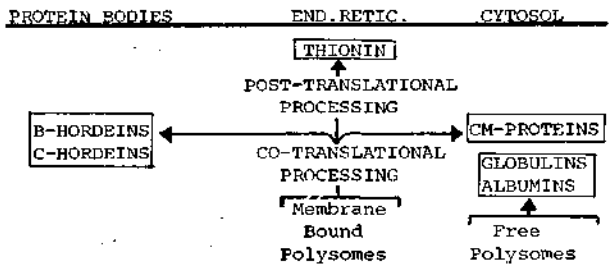

Fig. 5 Pathways of protein synthesis, processing and deposition in barley endosperm

Figure 5 summarizes the reported pathways of protein synthesis, processing and deposition in barley endosperm: i) synthesis of many cytosol albumins and globulins by free polysomes (BRANDT and INGVERSEN 1976); ii) synthesis of CM-proteins by membrane bound polysomes, with co-translational processing, and export into the cytosol; iii) synthesis of B, C, and D-hordein by membrane-bound polysomes, with co-translational excision of signal peptides, and deposition in

protein bodies; iv) synthesis of thionins by membrane-bound polysomes, with co-translational and post-translational processing, and deposition as extrinsic membrane proteins.

Molecular cloning

It is evident that the availability of c-DNA and genomic clones of cereal endosperm proteins would greatly enhance our capacity to carry out studies in wide-ranging areas of the basic and applied biology of this tissue, such as genome structure, gene expression, genetic modification of quality-related characters, manipulation of agronomic traits, etc. Our general aim at this level was to obtain c-DNA and genomic clones for the most relevant proteins alluded in this review. Our initial results concern the molecular cloning of c-DNA corresponding to the CM-proteins and thionins from barley. The following standard steps were followed: a) Preparation of total polysomal RNA from barley endosperm collected at about 20 d after anthesis. b) Purification of poly A^+RNA by affinity chromatography (oligo-dT cellulose column). c) Synthesis of double stranded c-DNA, using the poly A^+RNA as template. d) Tailing of the ds c-DNA with poly C. e) Selecting tailed c-DNA molecular greater than $\sim$350 bp long. f) Annealing with PstI restricted, poly G tailed pBR 322. g) Cloning into *E. coli* MC 1601. h) Selection of tetracycline resistant, ampicillin sensitive clones. i) Screening of the selected clones by hybridization with a c-DNA probe prepared from an RNA sucrose gradient fraction of poly A^+RNA from membrane bound polysomes, enriched in mRNAs for thionins and CM-proteins. j) Screening of clones selected in the previous step by hybrid release translation and identification of *in vitro* translation products with monospecific antibodies.

Clones obtained as described are being characterized and used as probes to select genomic clones.

Zusammenfassung

Chromosomale Lokalisierung und Expression von Genen für Proteine mit niedrigem Molekulargewicht in Weizen und verwandten Arten

Es wird ein Überblick über neuere Arbeiten zur chromosomalen Lokalisierung und Expression von Genen für Endospermproteine aus Weizen, Gerste und Roggen gegeben, die wahrscheinlich nicht zu den Reserveproteinen gehören und in mäßiger Menge vorliegen. Verwandte Aspekte, wie regulatorische genetische Effekte, *in vivo*- und *in vitro*-Synthese, Arten des processing, Speicherorte und molekulare Klonierung, werden ebenfalls diskutiert. Die Bedeutung dieser Untersuchungen für die Biologie des Endosperms, die genetische Manipulation von Qualität und agronomischen Eigenschaften und die Evolution dieser wichtigen Getreidearten wird kurz behandelt.

Краткое содержание

Хромосомальная локализация и экспрессия генов, регулирующих протеины с низким молекулярным весом у пшеницы и родственных видов

Дается обзор новых работ о хромосомальной локализации и экспрессии генов, регулирующих протеины эндосперма пшеницы, ячменя и ржи, которые, вероятно не являются запасными белками и встречаются в небольших количествах. Обсуждаются также близкие аспекты, как регулирующие генетические эффекты, синтез in vivo и in vitro, способ химической обработки, места запасания и молекулярное клонирование. Кратко рассматриваются значение этих исследований для биологии эндосперма, генетичесике манипуляции качества и агрономических особенностей, а также эволюция этих важных видов зерновых злаков.

Literature

Aragoncillo, C., M. A. Rodriguez-Loperena, P. Carbonero and F. Garcia-Olmedo, 1975: Chromosomal control of non-gliadin proteins from the 70 % ethanol extract of wheat endosperm. — Theor. Appl. Genet. **45**, 322–326.

—, —, G. Salcedo, P. Carbonero and F. Garcia-Olmedo, 1978: Influence of homoeologous chromosomes on gene-dosage effects in allohexaploid wheat (*Triticum aestivum* L.) — Proc. Natl. Acad. Sci. **75**, 1446–1450.

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

Bozzini, A., P. Cantagalli and S. E. Piazzi, 1971: Chromosomal location of the genetic control of two proteins specifically attributed to hexaploid wheat by immunological methods. E.W.A.C. Newsl. 16–17.

Brandt, A., and J. Ingversen, 1976: *In vitro* synthesis of barley endosperm proteins on wild type and mutant templates. Carlsberg Res. Commun. **41**, 311–320.

—, and —, 1978: Isolation and translation of hordein messenger RNA from wild type and mutant endosperms in barley. — Carlsberg Res. Commun. **43**, 451–469.

Burr, B., F. A. Burr, I. Rubenstein and M. N. Simon, 1978: Purification and translation of zein messenger RNA from maize endosperm protein bodies. — Proc. Natl. Acad. Sci. **75**, 696–700.

Caldwell, K. A. and D. D. Kasarda, 1978: Assessment of genomic and species relationships in *Triticum* and *Aegilops* by PAGE and by differential staining of seed albumins and globulins. — Theor. Appl. Genet. **52**, 273–280.

Carbonero, P. and F. Garcia-Olmedo, 1969: Purothionins in *Aegilops-Triticum* spp. — Experientia **25**, 1110.

Cubadda, R., 1975: Chromosomal location of genes controlling the synthesis of some soluble proteins in *T. diurum* and *T. aestivum*. In: Genetics and Breeding of Durum Wheat (ed. G. T. Scarascia-Muguozza). Library of the Faculty of Agric., Bari, and Natl. Inst. Nutr., Rome, 653–659.

Delibes, A., F. Dosba, C. Otero and F. Garcia-Olmedo, 1981: Biochemical markers associated with two M^v chromosomes from *Aegilops ventricosa* in wheat-*Aegilops* addition lines. — Theor. Appl. Genet. **60**, 5–10.

Doekes, G. J., 1968: Comparison of wheat varieties by starch gel electrophoresis of their grain proteins. — J. Sci. Food Agric. **19**, 169–176.

Doll, H. and A. H. D. Brown, 1979: Hordein variation in wild (*Hordeum spontaneum*) and cultivated (*H. vulgare*) barley. — Can. J. Genet. Cytol. **21**, 391–404.

Donovan, G. R., J. W. Lee and T. J. Longhurst, 1982: Cell-free synthesis of wheat prolamins. — Aust. J. Plant Physiol. **9**. 59–68.

Doussinault, G., A. Delibes, R. Sanchez-Monge and F. Garcia-Olmedo, 1983: Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. — Nature **303**, 698–700.

FERNANDEZ DE CALEYA, R., C. HERNANDEZ-LUCAS, P. CARBONERO and F. GARCIA-OLMEDO, 1976: Gene expression in alloploids: genetic control of lipopurothionins in wheat. – Genetics **83**, 687–699.

FRA-MON, P., G. SALCEDO, G. ARAGONCILLO and F. GARCIA-OLMEDO, 1984: Chromosomal assignment of genes controlling salt-soluble proteins (albumins and globulins) in wheat and related species. – Theor. Appl. Genetic, in press.

GARCIA-OLMEDO, F. and P. CARBONERO, 1970: Homoeologous protein synthesis controlled by homoeologous chromosomes in wheat. – Phytochemistry **9**, 1495–1497.

–, –, C. ARAGONCILLO, R. FERNANDEZ DE CALEYA and J. V. TORRES, 1976: Expression of homoeologous molecular systems in wheat alloploids. In: Heterosis in Plant Breeding (ed. A. JANOSSY and F. G. H. LUPTON, Proc. 5th Eucarpia Congress. Akadémiai Kiadó, Budapest, 51–57.

–, – and B. L. JONES, 1982: Chromosomal locations of genes that control wheat endosperm proteins. – In: Advances in Cereal Science and Technology **5**, (ed. Y. POMERANZ) 1–47.

– and R. GARCIA-FAURE, 1969: A new method for the estimation of common wheat (*T. aestivum* L.) in pasta products. – Lebensm. Wiss. Technol. **2**, 94–96.

GREENE, F. C., 1981: *In vitro* synthesis of wheat (*Triticum aestivum* L.) storage proteins. – Plant Physiol. **68**, 778–783.

HALL, O., B. L. JOHNSON and R. OLERED, 1966: Evaluation of genome relationships in wheat from their protein homologies. – Hereditas, Suppl. **2**, 47–54.

HART, G. E., D. E. MCMILLIN and E. R. SEARS, 1976: Determination of the chromosomal location of a glutamate oxalacetate transaminase structural gene using *Triticum-Agropyron* translocations. – Genetics **83**, 49–61.

–, A. K. H. R. ISLAM and K. M. SHEPHERD, 1980: Use of isozymes as chromosome markers in the isolation and characterization of wheat-barley chromosome addition lines. – Genet. Research **36**, 311–325.

HEJGAARD, J., 1982: Purification and properties of protein Z a major albumin of barley endosperm. – Physiol. Plant. **54**, 174–182.

JOHNSON, B. L., 1972: Protein electrophoretic profiles and the origen of the B genome of wheat. – Proc. Natl. Acad. Sci. **69**, 1398–1402.

–, 1975: Identification of the apparent B genome donor of wheat. – Can. J. Genet. Cytol. **17**, 21–39.

–, and O. HALL, 1965: Analysis of phylogenetic affinities in the Triticinae by protein electrophoresis. – Am. J. Bot. **52**, 506–513.

LARKINS, B. A. and W. J. HURKMAN, 1978: Synthesis and deposition of zein in protein bodies of maize endosperm. – Plant Physiol. **62**, 256–263.

LUTHE, D. S., and D. M. PETERSON, 1975: Cell-free synthesis of globulin by developing oat (*Avena sativa* L.) seeds. – Plant Physiol. **56**, 256–263.

MATLASHEWSKI, G. J., K. ADELI, I. ALTOSAAR, P. R. SHEWRY and B. J. MIFLIN, 1982: *In vitro* synthesis of oat globulin. – FEBS Lett. **145**, 208–212.

MATTHEWS, J. A. and B. J. MIFLIN, 1980: *In vitro* synthesis of barley storage proteins. – Planta **149**, 262–268.

MIFLIN, B. J., and P. R. SHEWRY, 1979: The biology and biochemistry of cereal seed prolamins. – In: Seed protein improvement in cereals and grain legumes. Proc. Symp. IAEA/FAO. IAEA, Vienna, 137–158.

MIFLIN, B. J., and P. R. SHEWRY, 1979: The synthesis of proteins in normal and high lysine barley seeds. In: Recent Advances in the Biochemistry of Cereals (eds. D. LAIDMAN and R. G. WYN JONES). Academic Press, 234–273.

MOLINA-CANO, J. L., P. FRA-MON, G. SALCEDO, C. ARAGONCILLO and F. GARCIA-OLMEDO. – (in preparation).

NODA, K., and K. TSUNEWAKI, 1972: Analysis of seed proteins in ditelosomes of common wheat. Japan J. Genet. **47**, 315–318.

OKITA, T. W., and F. C. GREENE, 1982: Isolation and characterization of the gliadin messenger RNAs. – Plant Physiol. **69**, 834–839.

PAZ-ARES, J., C. HERNANDEZ-LUCAS, G. SALCEDO, C. ARAGONCILLO, F. PONZ and F. GARCIA-OLMEDO, 1983a: The CM-proteins from cereal endosperm: Immunochemical relationships. – J. Exp. Bot. **34**, 388–395.

– Paz-Ares, J., F. Ponz, C. Aragoncillo, C. Hernandez-Lucas, G. Salcedo, P. Carbonero and F. Garcia-Olmedo, 1983b: *In vivo* and *in vitro* synthesis of CM-proteins (A-hordeins) from barley (*Hordeum vulgare* L.). – Planta **157**, 74–80.

Ponz, F., C. Hernandez-Lucas, P. Carbonero and F. Garcia-Olmedo, 1984: Lipid binding proteins from the endosperms of wheat and oat. – Phytochemistry, **23**, 2179–2181.

–, J. Paz-Ares, C. Hernandez-Lucas, P. Carbonero and F. Garcia-Olmedo, 1983: Synthesis and processing of thionin precursors in developing endosperm from barley (*Hordeum vulgare* L.). – EMBO J. **2**, 1035–1040.

Powling, A., A. K. M. R. Islam and K. W. Shepherd, 1981: Isozymes in wheat-barley hybrid derivative lines. – Biochem. Genet. **19**, 237–254.

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

Rahman, S., P. R. Shewry and B. J. Miflin, 1982: Differential protein accumulation during barley grain development. – J. Exp. Bot. **33**, 717–728.

Rhodes, A. P., and A. A. Gill, 1980: Fractionation and amino acid analysis of the salt-soluble protein fractions of normal and high-lysine barleys. – J. Sci. Food Agric. **31**, 467–473.

Rodriguez-Loperena, M. A., C. Aragoncillo, P. Carbonero and F. Garcia-Olmedo, 1975a: Heterogeneity of wheat endosperm proteolipids (CM-proteins). – Phytochemistry **14**, 1219–1223.

–, –, J. V. Torres, P. Carbonero and F. Garcia-Olmedo, 1975b: Biochemical evidence of chromosome homoeology among related plant genera. – Plant Sci. Lett. **5**, 387–393.

Salcedo, G., C. Aragoncillo, M. A. Rodriguez-Loperena, P. Carbonero and F. Garcia-Olmedo, 1978: Differencial allelic expression at a locus encoding an endosperm protein in tetraploid wheat *(T. turgidum)*. – Genetics **89**, 147–156.

–, P. Fra-Mon, J. L. Molina-Cano, C. Aragoncillo and F. Garcia-Olmedo. Genetics of CM-proteins (A-hordeins) in barley. – Theor. Appl. Genet. **68**, 53–59.

–, J. Prada and C. Aragoncillo, 1979: Low molecular weight gliadin like proteins from wheat endosperm. – Phytochemistry **18**, 725–727.

–, –, R. Sanchez-Monge and C. Aragoncillo, 1980a: Aneuploid analysis of low molecular weight gliadins from wheat. – Theor. Appl. Genet. **56**, 65–69.

–, R. Sanchez-Monge and C. Aragoncillo, 1982: The isolation and characterization of low molecular weight hydrophobic salt soluble proteins from barley. – J. Exp. Bot. **33**, 1325–1331.

–, –, A. Argamenteria and C. Aragoncillo, 1980b: The A-hordeins as a group of salt soluble hydrophobic proteins. – Plant Sci. Lett. **19**, 109–119.

Sanchez-Monge, R., A. Delibes, C. Hernandez-Lucas, P. Carbonero and F. Garcia-Olmedo, 1979: Homoeologous chromosomal location of the genes encoding thionins in wheat and rye. – Theor. Appl. Genet. **54**, 61–63.

Shewry, P. R., H. M. Pratt, A. J. Faulks, S. Parmar and B. J. Miflin, 1979: The storage protein (hordein) polypeptide pattern of barley (*Hordeum vulgare* L.) in relation to varietal identification and disease resistance. – J. Nat. Inst. Agric. Bot. **15**, 34–50.

Viotti, A., E. Sala, R. Marotta, P. Alberi, C. Balducci and C. Soave, 1979: Genes and mRNAs coding for zein polypeptides in *Zea mays*. – Eur. J. Biochem. **102**, 211–222.

Waines, J. G., 1973: Chromosomal location of genes controlling endosperm protein production in *Triticum aestivum* cv. Chinese Spring. Proc. 4th Int. Wheat. Genet. Symp. (eds. E. R. Sears and L. M. S. Sears) Agric. Exp. Stn., Univ. of Missouri, Columbia, Mo. 873–877.

Wienand, U., and G. Feix, 1978: Electrophoretic fractionation and translation *in vitro* of poly (A)-containing RNA from maize endosperm. – Eur. J. Biochem. **92**, 605–611.

Yamagata, H., K. Tanaka and Z. Kasai, 1982: Biosynthesis of storage proteins in developing rice seeds. – Plant Physiol. **70**, 1094–1100.

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