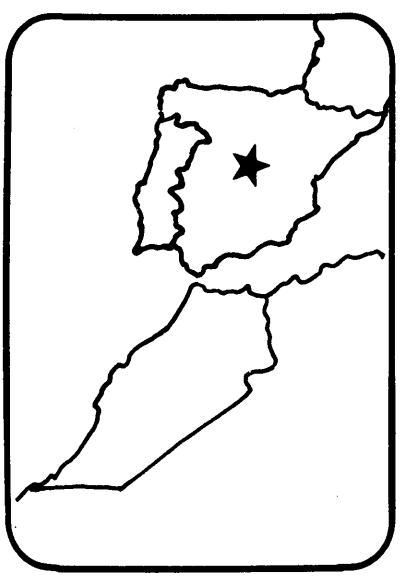
MP 41

3rd

INTERNATIONAL
WHEAT CONFERENCE

PROCEEDINGS . .

MADRID, SPAIN
May 22 - June 3
1980



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CONTRACT NO. AID/DSAN-G-0171

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CHROMOSOMES, GENES, PROTEINS AND WHEAT ENDOSPERM

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Summary

Data on the redundancy and expression levels of genes encoding wheat endosperm proteins are discussed in connection with their evolution and with the evolution of reserve proteins in other cereal crops. The molecular basis of the D-genome contribution to baking quality are analysed in terms of proteins and lipids.

Introduction

Our research group has been involved for some years in a study of the genetic control of the biochemical composition of wheat endosperm. The purpose of this contribution is to review those aspects of this work that are related in a general way to both the nutritional and the technological quality of wheat.

To a great extent, the genetic analysis has been made possible by the development of a great variety of aneuploids, which was pioneered by E. R. Sears (1,2). It should be pointed out that with aneuploids, the genetic analysis only goes down, at most, to the level of large chromosomal segments and that to actually deal with genes, data on segregation of allelic variants is required. These data are mostly lacking in the case of endosperm biochemical characters. In the case of some major protein components, this lack has been compensated in part by a thorough characterization of the gene products, but in the case of regulatory or quantitative effects, aneuploid analysis does not allow to discriminate between single gene and whole chromosome. Furthermore, it is often difficult to distinguish between true chromosomal effects on the level of expression of a certain biochemical character and the indirect consequences of deleting large segments of genetic information. In other words, it is difficult to tell apart relevant information about the regulation of gene expression from irrelevant, non-specific, drastic effects brought about by large deletions.

With these considerations in mind, we have chosen to discuss two specific aspects of the general topic which are respectively related to the nutritional and the technological quality of wheat: i) the genetic control of wheat endosperm proteins; and ii) the contribution of the D genome to baking quality.

Redundancy and Expression Levels of Genes Encoding Endosperm Proteins

Breadwheat (Triticum aestivum L., genomes AABBDD) is an allohexaploid in which most of its genetic information must have been triplicate when the species originated. Our early observations on

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the distribution of certain biochemical systems among species of the Aegilops-Triticum group suggested that redundant genetic expression is partially lost after poliploid formation (3,4). Subsequently, this phenomenon has been well demonstrated both in animals (see ref. 5) and in plants (6).

Data on the chromosomal location of genes that control different biochemical systems, obtained by aneuploid analysis in several laboratories, including ours, permit not only the estimation of the percentage of gene triplication and duplication expressed, but also to discern where the presumed "losses of redundacy", or inactivations, have taken place. We have carried out such a survey (6) and out of 28 sets of homoeologous systems, 16 were controlled by triplicate loci, 7 by duplicate loci, and 5 by single loci (Table 1). The proportions of silenced loci turned out to be different for each genome. A (32%) > B (18%) > D (11%). Most of the incomplete homoeologous sets were associated with major endosperm proteins with no apparent enzymatic function, whereas most of the enzymatic loci were triplicate (Table 1). This means, in practical terms, that the genetic systems for wheat endosperm proteins seem to be far more diploidized than the rest of the genetic information in wheat.

Table 1. Redundancy of genes encoding biochemical markers in wheat.

Redundancy	No of sets	%	Genomes A	B	D	Type of system
triplicate	16	57	+	+	+	Mostly enzymes
duplicate	7	25	+	+	-	
			+	-	+	Mostly endosperm
			-	+	+	proteins of presumed
single	5	18	+	-	-	reserve function
			-	+	-	
			-	-	+	
% silenced loci			32	18	11	

From a theoretical point of view it is of interest to speculate about the implications of such sharp contrast. Loss of redundant gene expression after polyploid formation is the simplest but not the only plausible explanation of our failure to observe triplicate loci for major endosperm proteins.

Alternatively, genes for some of the reserve proteins might have been silenced in some of the diploid species prior to the alloploid formation. This would imply that the expression of such genes might not be vital for the plant and their failure to express themselves could be compensated by an increased expression of genes encoding other non-homologous reserve proteins.

The fact that allopoid protein patterns can be often reconstructed by mixing in the test tube the diploid genome donors could be considered as evidence against the first alternative, since this would indicate that no extensive loss of expression of genes expressed in the genome donors could have taken place in the alloploid.

The second hypothesis could be further used to explain the striking diversity of reserve proteins among cultivated cereals. Most of these proteins do not seem to be as closely related from a structural point of view as would be expected of proteins encoded by genes derived from a close common ancestor, as is certainly the case for proteins with known enzymatic function. A model for the evolution of reserve proteins, based in the above is presented in Figure 1.

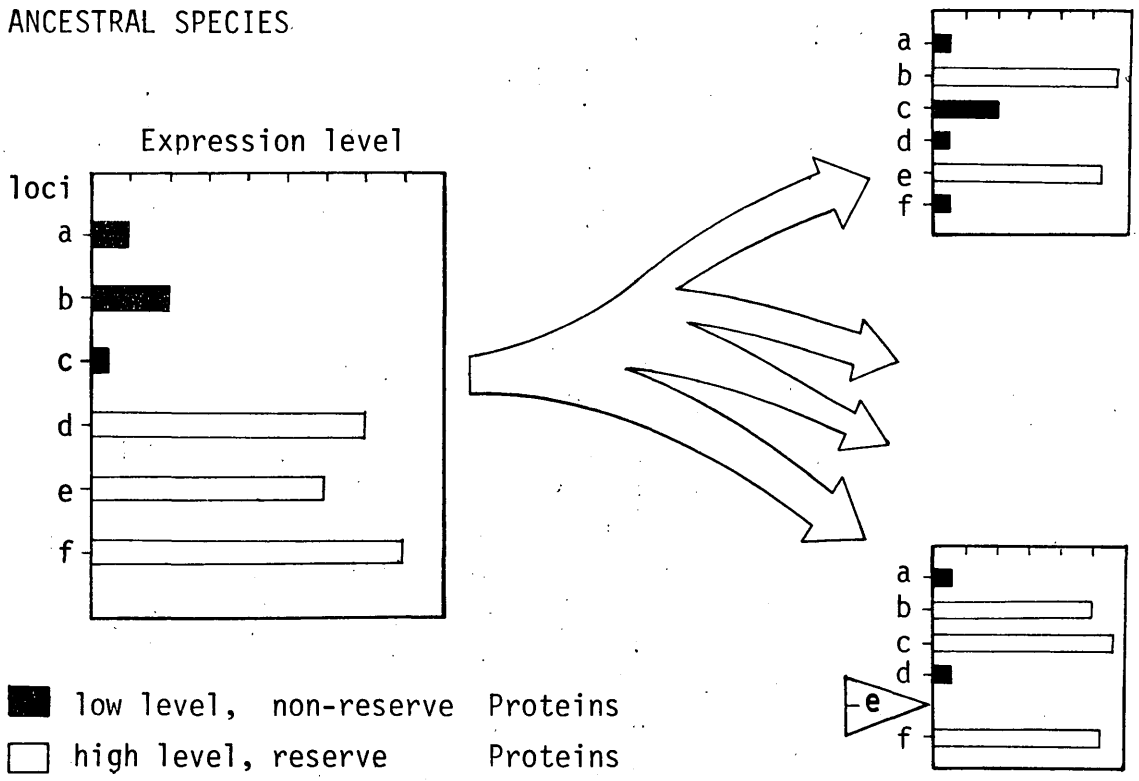

Figure 1. Model for the evolution of reserve proteins in cereal.

We have shown that the levels of certain individual protein components are strictly determined by gene dosage, while for other proteins, the net output at each dosage of its structural gene, can be 30-80% higher when the chromosome carrying an active homoeogene was absent (7). This is exemplified in Figure 2.

The amount of protein per gene dose, in the case of proteins of the first class, seems to be genetically determined, as has been shown in the case of the allelic pair CM3/CM3' (8). The amount of CM3 accumulated per gene dose, on a molar basis, is about twice that of CM3' (Figure 3). The genetic evidence indicates that the quantitative difference either depends on differences in the structural gene itself or is controlled by regulatory or modifier gene(s) linked to it.

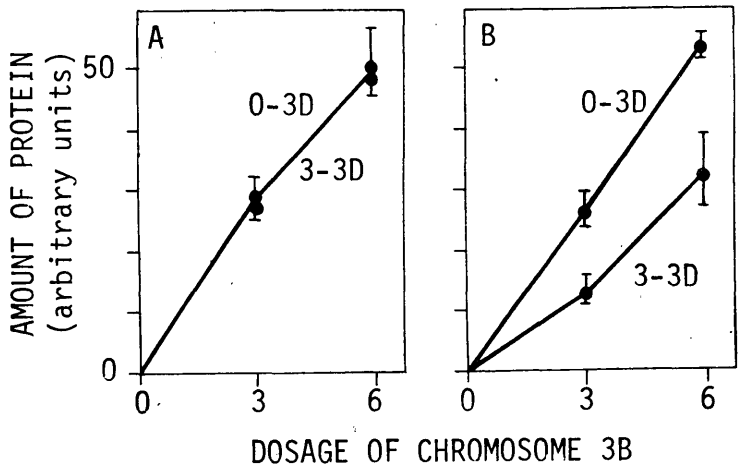

Figure 2. Gene-dosage responses of two proteins encoded by genes located in chromosome 3B. A) Protein in which gene-dosage response is not affected by chromosome 3D (presence or absence). B) Protein that is at a higher level in the absence of chromosome 3D, which carries a homoeogene (Data taken from ref. 7).

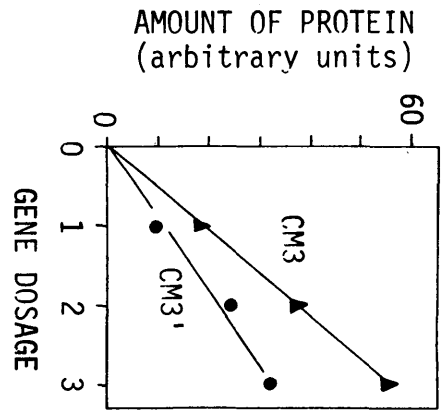

Figure 3. Gene-dosage responses of two allelic proteins, CM3/CM3' in T. durum endosperm. (Data taken from ref. 8).

The two cases just considered of alteration of gene-dosage responses would respectively represent examples of the short-term and long-term genetic mechanisms that would make possible the evolution of reserve proteins according to the model in Figure 1.

The well known high-lysine mutants described in other cereal crops can also be considered to reveal compensatory mechanisms of the type required by the model, as their effects can be interpreted as an enhancement of the expression of genes encoding certain lysine-rich proteins brought about by the partial blockade of the expression of genes encoding certain prolamines (zeins in maize, hordeins in barley).

Molecular Basis of the D-genome Contribution to Baking Quality

It is generally accepted that gluten proteins, gliadins and glutenins, play an important role in relation to baking quality and it has been assumed that the differences in baking quality between hexaploid and tetraploid wheats were mainly due to gluten proteins contributed by the D genome. Indeed, genes encoding gliadins and glutenins have been located in D-genome chromosomes by aneuploid analysis (see ref. 9).

The importance of wheat lipids in the above context has been often overlooked. Pomeranz and coworkers (10) found that bread volume was significantly decreased if "free" lipids were extracted from flour with petroleum ether. By adding back to the flour different fractions from the extract, it was found that the polar fraction was effective in restoring the original bread volume, whereas the non-polar was not. By subfractionation of the polar fraction, it was possible to identify digalactosyl diglyceride (DGDG) as the lipid class whose extraction determined the volume loss. Studies by infrared and nuclear magnetic resonance spectroscopy and by autoradiography seem to indicate that DGDG improves the retention of fermentation gases due to its ability to form hydrogen bonds with starch and gliadins and hydrophobic bonds with glutenins.

We found a sharp difference in the amounts of DGDG present in T. aestivum as compared with T. durum cultivars (Table 2, exp. 1). The extraction of the D genome from a hexaploid cultivar markedly decreased DGDG and the addition of the D genome to a tetraploid cultivar resulted in an enhanced DGDG level (Table 2, exp. 2). Finally, it was established by aneuploid analysis that a gene or genes located in the short arm of chromosome 5D was associated with the observed interspecific difference (Table 2, exp. 3).

These results strongly suggest that the addition of the D genome altered the baking properties of tetraploid wheats not only by altering their protein composition but also by drastically changing their polar-lipids profile (11).

Table 2. Relative contents of digalactosyl diglyceride (DGDG) in different wheat stocks.

Experiment no.	Stock	DGDG mm^2*	
1	T.aestivum cvs		
	Cabezorro	385	
	Pané 247	204	
	Chinese Spring	180	
	Aragón 03	176	
	Rieti	275	
	F.Aurora	282	
	Dimas	169	
	Ariana	88	
	Libero	172	
	Mara	173	
	Roma	196	
	Impeto	264	
			215 av.
	Composite flour A (37 cvs)	212	
	Composite flour B (5 cvs)	180	
	T.durum cvs		
	Hibrido D	12	
	Alaga	34	
	S.Capelli	72	
	Jerez 36	8	
	Andalucia	23	
			30 av.
	Composite flour C (5 cvs)	60	
2	Thatcher (ABD)	250	
	Tetra-Thatcher (AB)	45	
	Ae.squarrosa (D)	280	
	T.carthlicum (AB)	30	
	T.spelta (ABD)	190	
3	T.aestivum cv. Chinese Spring	185	
	ditelo 5DL	20	
	nulli 5D-tetra 5A	35	
	nulli 5A-tetra 5D	300	

*Densitometric determinations. Comparisons are valid within each experiment.

Final Remarks

Biochemists deal with specific molecules (proteins, lipids, etc.) and breeders try to manipulate global chemical composition either directly or through quality-related parameters. It is of interest to integrate both views. It is true that sometimes the trees do not allow to see the forest, but it is no less true, in our opinion, that proper management of the forest may require at times to pay attention to the individual trees. The present contribution is a modest and brief attempt of integration, taking examples from our own work as biochemists.

Acknowledgements

We thank Drs. C. Aragoncillo, G. Salcedo and C. Hernandez-Lucas for discussions and acknowledge grants from the Areces Foundation and the Comision Asesora de Investigacion Cientifica y Tecnica.

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