# Expression of homoeologous molecular systems in wheat alloploids

F. GARCÍA-OLMEDO, P. CARBONERO, C. ARAGONCILLO, R. FERNÁNDEZ DE CALEYA AND J. V. TORRES

*Department of Biochemistry, Polytechnical University of Madrid*
*E. T. S. Ingenieros Agrónomos*
*Madrid-3, Spain*

SUMMARY

Allopolyploidy is widespread in the plant kingdom, where it has been of considerable evolutionary significance. Although the existence of heterotic interactions between the genomes that make up an alloploid have been generally assumed, the precise nature of these interactions has not been extensively investigated.

Presently available evidence about metabolic integration of the wheat genomes is examined in search of new insights about the different modes of genome interaction. Although additive expression seems to be the case for many homoeologous systems, more complex patterns of integration have become evident. Examples of enzyme subunit complementation, gene compensation and other dosage effects, holoprotein completion, and complementation of metabolic pathways are discussed.

## INTRODUCTION

One of the most important processes which have affected the evolution of higher plants is allopolyploidy. An alloploid is in fact a "permanent heterozygote" in which positive and negative heterotic interactions between homoeoalleles are effectively fixed.

It is accepted that, in terms of measurement of specific characters, heterosis implies values outside the parental range. In the case of alloploids, inter-genome heterosis is difficult to assess because it is superposed with positive and negative effects associated with the change in ploidy level. It could be argued that in an allotetraploid the terms of reference should be the genome donors at the tetraploid level. This criterion would be hard to apply to the case of an allohexaploid. Characters controlled by only two homoeogenes could be investigated at the homozygous tetraploid level in the appropriate nulli-tetrasomics. In most cases, however, ploidy and heterosis effects have to be considered jointly.

A variety of theories have been put forward to explain heterosis. These can be generally grouped into dominance and overdominance theories or, in other words, those based on intergenic complementation and those based on interallelic complementation (see, for example Finchham, 1966). It has been acknowledged for a long time that the two types of theories are not mutually exclusive and that both types of phenomena are probably involved in the observed association of heterosis and heterozygosity.

The biochemical bases of heterosis are becoming better known. The studies with hybrid antigens and, more conclusively, those with hybrid proteins have clarified the nature of inter-allelic complementation; while numerous examples of complementation at the level of metabolic pathways have accumulated (for a review see Manwell & Baker, 1970).

The study of inter-genomic interactions should be easier to undertake at the molecular rather than at the morphological or physiological levels of gene action. The purpose of this paper is to examine available data about homoeologous biochemical systems in wheat alloploids in the above-mentioned context.

## METABOLIC INTEGRATION OF HOMOEOLOGOUS MOLECULAR SYSTEMS IN WHEAT

A number of homoeologous molecular systems have been studied in wheat. Those to be discussed here are listed in Table 1. The list includes homoeosystems which have been studied at the protein level, others described at the level of enzyme activities and finally a few characterized by their metabolic product profiles. Available

*Table 1.* Homoeologous molecular systems in wheat

| SYSTEMS | CHROMOSOMAL LOCATIONS |
|---|---|
| *Proteins* | |
| Glutenins | group 1 |
| Gliadins | groups 1 and 6 |
| CM-Proteins | groups 7 and 4 |
| Purothionins | group 1 |
| *Enzymes* | |
| Acid phosphatase | 4Aβ, 4BS, 4DL |
| Alcohol dehydrogenase | 4Aα, 4BL, 4DS |
| Alpha-amylase | 6Aβ, 6BL, 6Dβ<br>7AL, 7BL, 7DL |
| Aminopeptidase | 6Aα, 6BS, 6Dα |
| Esterase | 3Aβ, 3BS, 3Dα and β |
| Peroxidase | group 1 |
| *Metabolic products* | |
| Anthocyanins | group 7 |
| Phenolic glycosides | 5A, 6A, 2B, 3B |
| and glycoflavones | 5B, 1D, 7D |
| Sterol esters | group 7 |

quantitative data about their expression will give us some clues of the different ways in which they are metabolically integrated.

Early biochemical studies with alloploids showed that, in general, biosynthetic activity of the component genomes was maintained in them, although some proteins present in one or both parents were not detected (Hall, 1959). "Hybrid substances", present in the alloploid but absent in the genome donors, were looked for in these studies but not unequivocally demonstrated until later, when heterooligomeric en-

zymes were shown to exist. The impression of strictly additive metabolic activity was reinforced by observations of reserve seed protein by electrophoresis in ditelosomics and nulli-tetrasomics, where chromosome dosage effects were evident (Shepherd, 1968). This seems also to be the case for aminopeptidase and acid phosphatase (Hart, 1973).

However, when other systems have been studied, more complex patterns of genome interactions, such as enzyme subunit complementation, gene dosage compensation, holoprotein completion and metabolic complementation have been shown to exist.

## ENZYME SUBUNIT COMPLEMENTATION

The presence of hybrid enzymes in heterozygotes was first demonstrated by Schwartz (1960) for the $E_1$ esterase of maize. Some years later he presented evidence of single gene heterosis for maize alcohol dehydrogenase (1969). The $Adh_1F$ allele specifies an active but somewhat labile dimeric enzyme (FF) and the $Adh_1$ Cm allele produces a less active but more stable enzyme (Cm Cm). In the heterozygote a heterodimer (F Cm) is formed which is both active and stable.

Barber *et al.* (1968) obtained evidence of wheat esterase subunit structure and, more recently, Hart (1970) has demonstrated intergenomic heterodimers in wheat alcohol dehydrogenase. His results are consistent with the existence of one locus in each of the group 4 chromosomes that produce about equal amounts of their specific subunits ($\alpha, \beta, \delta$) which by random association generate the six possible dimers in the expected proportions ($^2/_9$ each heterodimer; $^1/_9$ each homodimer). This illustrates how additive genetic activity of the genomes can result in non additive functional effects. Indeed, additive electrophoretic patterns obtained under dissociating conditions do not rule out non-additive interactions in vivo.

## GENE DOSAGE COMPENSATION AND OTHER DOSAGE EFFECTS

Although the relative amounts of homoeoallelic products are very often proportional to the number of doses of the chromosome(s) carrying each homoeoallele, this is not always the case. At least two types of non additive dosage effects have been demonstrated: gene dosage compensation and inhibition of protein synthesis by extra doses of chromosomes non-homoeologous with that carrying the structural genes.

We have shown that two electrophoretic components of endosperm protein soluble in chloroform-methanol, CM1 and CM2, are controlled by chromosomes 7D and 7B respectively (García-Olmedo & Carbonero, 1970; Aragoncillo, 1973). Densitometric measurements of these components in different ditelosomic lines are shown in Fig.1. Suppression of CM1 synthesis in the appropriate ditelosomic is compensated by increased synthesis of CM2 and vice versa. However, suppression of CM1 synthesis by extraction of the whole D genome does not yield the same result. It seems that the level of this type of proteolipid determined by some regulatory factor associated with the D genome or resulting from genome interaction.

A similar situation has been reported by May *et al.* (1973) for the esterases controlled by group 6 chromosomes. Suppression of chromosome 6A esterase (Est-6AL-1) is compensated by increased synthesis of Est-6DL-1 and vice versa. However, Est-6BL-1 was not under the same type of regulation. This has been explained by postulating that the regulatory genes of Est-6AL-1 and Est-6DL-1 are unchanged while those of Est-6BL-1 have diverged. The alpha-amylases of groups 6 and 7 may be regulated in the same way (Nishikawa & Nobuhara, 1971).

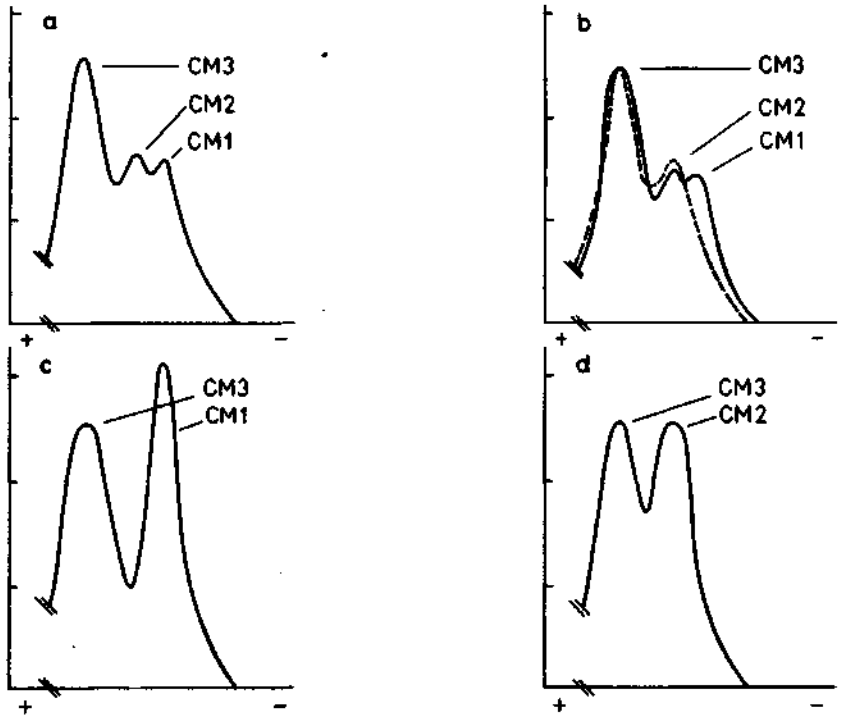


Fig. 1. CM-pattern densitograms of a) euploid Chinese Spring, b) Thatcher and tetra-Thatcher (– – –), c) ditelo 7BL Chinese Spring, d) ditelo 7DS Chinese Spring.

That extra doses of one chromosome can inhibit synthesis of proteins coded by genes located in a chromosome belonging to a different homoeologous group has been shown by Shepherd (1968) for the gliadins and by Orth & Bushuk (1973) for the glutenins. Four doses of chromosome 2A inhibited synthesis of chromosome 6D gliadins. In a similar way, four doses of either 2B, 3B, or 6B inhibited synthesis of certain glutenins coded by 1D.

## HOLOPROTEIN COMPLETION COMPLEMENTATION

An interesting case of genome interaction is that of lipopurothionin synthesis, presently under study in our laboratory (Carbonero & García-Olmedo, 1969; F. de Caleya *et al.*, in prep.). Purothionins are basic polypeptides that can be extracted with light petroleum from wheat endosperm while non covalently associated with polar

lipids, but are insoluble in this non polar solvent as the free apoproteins (Carbonero *et al.*, 1969; Fernández de Caleya *et al.*, in prep.; Balls *et al.*, 1942; Nimmo *et al.*, 1968; Fisher *et al.*, 1968; García-Olmedo *et al.*, 1968). Two electrophoretic variants of the apoprotein are known, named α and β.

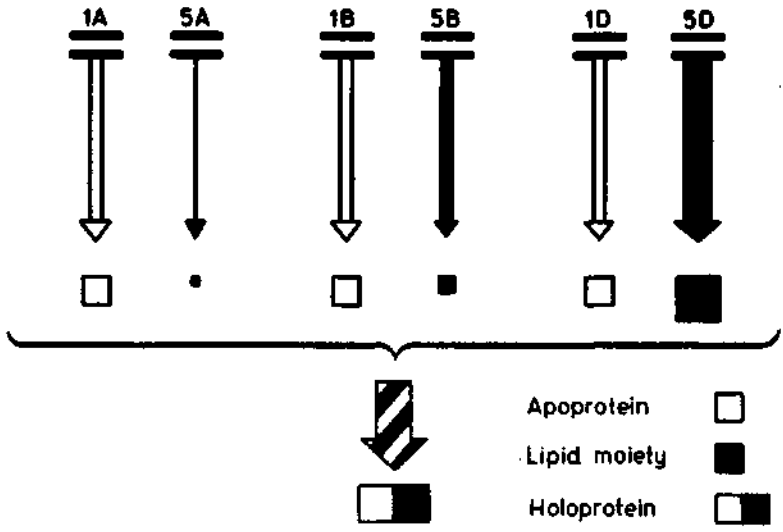


Fig. 2. Tentative model of genetic control of lipopurothionins synthesis.

Preliminary results of an electrophoretic analysis of the purothionin system are summarized in Tables 2 and 3. A tentative model based on these data is outlined in Fig. 2. The results are consistent with location of the apoprotein structural genes

*Table 2.* Amount of lipopurothionins in different genotypes

Euploid ~ n 5A t 5B ~ n 5A t 5D ≥ n 5B t 5D ≫ n 5B t 5A > n 5D t 5B > n 5D t 5A
hexaploid wheats ≫ tetraploids
synthetic *T. spelta* ≥ *Ae. squarrosa* ≫ *T. carthlicum*
Prelude ≫ tetra-Prelude
Rescue ≫ tetra-Rescue
Thatcher ≫ tetra-Thatcher

*Table 3.* Ratio of α and β purothionins in different genotypes

| | α/β |
|---|---|
| Tetraploid wheats | 1 |
| Hexaploid wheats | 2 |
| Euploid C.S. | 2 |
| nulli 1A – tetra 1B | no β |
| nulli 1A – tetra 1D | α enhanced |
| nulli 1B – tetra 1A | ½ |
| nulli 1B – tetra 1D | 2 |
| nulli 1D – tetra 1A | ½ |
| nulli 1D – tetra 1B | 2 |
| Other nulli-tetrasomics | 2 |

in homoeologous chromosome group 1 and of some regulatory factors, possibly affecting lipid moiety synthesis, in group 5. The gene for β purothionin would be located in chromosome 1A and two genes for α purothionin would be located in 1B and 1D respectively. While the three apoprotein genes seem to be about equally productive, and thus fully compensate for each other in the nulli-tetrasomics, this is not the case for the genes located in group 5. The results in Table 2 indicate non-compensation. The three homoeologous chromosomes could be ranked as to their affectiveness in lipopurothionin production as follows: 5D > 5B ⩾ 5A.

A more complete account of this research is in preparation.

## COMPLEMENTATION OF METABOLIC PATHWAYS

Evidence of intergenomic complementation can be derived from the study of metabolic product profiles as will be illustrated in the following discussion.

One such a case is the regulation of sterol metabolism, which has been studied in our laboratory (García-Olmedo, 1968; Torres & García-Olmedo, 1968; 1974, and in press). The sterol esters pattern of *T. aestivum* endosperm is different from that of *T. durum:* palmitate is the dominant ester in the first species and linoleate in the second. *Ae. squarrosa* pattern resembles that of *T. aestivum.* A few *T. aestivum* varieties show a pattern like that of *T. durum.* This phenotypic difference is controlled by a single Mendelian factor (García-Olmedo, 1968), which is located in chromosome 7DS (Torres & García-Olmedo, 1974). Palmitate synthesis takes place in the late stages of endosperm development and drains the high level of free sterol that exists in the early stages of development. The recessive allele determines blockage of palmitate synthesis, high level of free sterol in the mature kernel, and some accumulation of steryl glycosides (Torres & García-Olmedo, in preparation). Data in Table 4 show that the activity of the dominant allele is high enough to cause the esterification of sterol resulting not only from D genome activity but also from that of genomes A and B. No dosage effect is observed.

Gale & Flavell (1971) have studied the genetic control of anthocyanin biosynthesis using Hope-Chinese Spring substitution lines and their hybrids. They found homoeologous loci in chromosomes 7A and 7B that controlled flavonoid class profiles. In this case a linear chromosome dosage effect was demonstrated.

May *et al.* (1973) have tentatively concluded that the two-step transformation of the o-glycoflavone saponarin into wyomin is controlled by two genes of chromosome 5A. This system would probably behave in the same way as that of the sterol esters.

*Table 4.* Regulation of sterol palmitate and free sterol in wheat endosperm

| Material | | Free sterol* | Sterol palmitate* |
|---|---|---|---|
| *T. aestivum* | var. Aragon 03 | 3.3 | 11.8 |
| *T. aestivum* | var. Mara | 21.3 | 1.7 |
| *T. aestivum* | var. Chinese Spring | 1.4 | 6.3 |
| *T. aestivum* | var. C.S. nulli 7D tetra 7A | 4.0 | 0.1 |
| *T. aestivum* | var. C.S. nulli 7D tetra 7B | 4.0 | 0.3 |
| *T. durum* | var. Senatore Capelli | 5.6 | 0.6 |

* mg of sterol per 100 g dry matter.

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