

CHROMOSOMAL LOCATIONS OF GENES THAT CONTROL WHEAT ENDOSPERM PROTEINS

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I. INTRODUCTION

The nutritional value and the unique dough-forming and baking properties of wheat flour largely depend on its protein content and composition. Wheat endosperm has poor nutritional quality because it is deficient in certain essential amino acids (especially lysine), but because it is abundant, it is still the single most important source of protein for much of the world's population. Although wheat proteins have been extensively investigated by biochemists, geneticists, and breeders for well over two centuries, our knowledge about them is limited, compared with that of other important sources of dietary proteins, such as meat or milk. Only during the past 15 years have significant advances been made in the biochemical and genetic studies of individual wheat protein components. Most of the genetic studies have been on the aneuploid lines developed and made available by Sears (1953, 1954, 1959, 1966). Progress in aneuploid analysis of genes controlling proteins has generally depended on advances in protein fractionation and characterization. In many cases, however, these genetic analyses have been done on insufficiently characterized groups of proteins, and this has resulted in some confusion.

II. SOME RELEVANT ASPECTS OF WHEAT GENETICS

Before discussing the genetic control of specific groups of endosperm proteins, it seems pertinent to provide a brief account of our knowledge about the genome organization of the cultivated wheats, the extensive array of special stocks currently used in the genetic analyses of wheat proteins, and the limitations of such genetic analyses.

A. Genome Complements of Cultivated Wheats

Commercially cultivated wheats are allopolyploids, which are also called allopolyploids and amphiploids. Allopolyploids are species that originate by hybridization and chromosome duplication of two genetically isolated parental species that evolve divergently from a common ancestor (Fig. 1). Chromosome duplication occurs naturally with a very low frequency that can be increased by colchicine treatment of the interspecific hybrids and by other procedures.

The term "homoeology," or ancestral homology, designates the homology relationships between genomes, chromosomes, or genes derived from a common

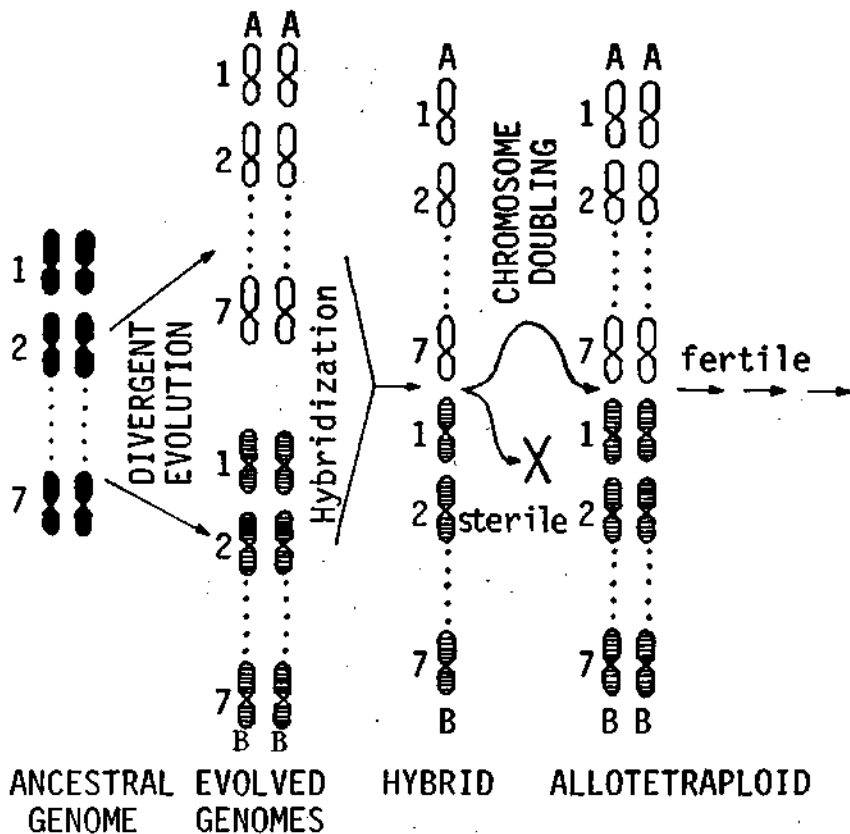


Figure 1. Origin of an allopolyploid. Genomes A and B have evolved into distinct species from a common ancestral genome, and their hybrids are sterile unless chromosome doubling occurs. The new allotetraploid species behaves as a functional diploid, is fully fertile, and is at least partially isolated from the genome donors. Genomes A and B are homoeologous. Chromosomes are numbered to indicate homoeology relationships (e.g., 1A-1B).

ancestor (e.g., genomes A and B, chromosomes 1A and 1B in Fig. 1).

The genome structures and the origins of cultivated wheats have been investigated by cytological examination of hybrids between the wheats and their wild relatives, by cytological and phenotypic comparisons of the wheats with synthetic allopolyploids, and by comparisons of the biochemical constituents of the putative genome donors with those of the present wheats. Excellent reviews on this subject have recently been written by Sears (1975, 1977a) and by Feldman (1976, 1979).

Durum or macaroni wheat, *Triticum turgidum* L. var. durum (Desf.), is an allotetraploid containing two sets (seven pairs each) of chromosomes (genomes AABB). Common or bread wheat, *T. aestivum* L. var. aestivum, is an allohexaploid that has an additional set of seven pairs of chromosomes (genomes AABBDD). The wild diploid species that donated the A and the D genomes to the cultivated wheats have been identified beyond reasonable doubt, but there is still controversy concerning the possible B genome donor(s). Genome A was contributed by a wild form of the diploid wheat *T. monococcum* L., which participated in the cross that resulted in the primitive form of the tetraploid wheat, *T. turgidum* L. This species was cultivated in the Near East as long as 10,000 years ago. Forms of the cultivated emmer with nonfragile rachis were derived; These are the origin of the present free-threshing durum wheats. Eventually, a spontaneous, nonfertile hybrid between the cultivated emmer and the weed *Aegilops squarrosa* L. (syn. *T. tauschii* (Coss.) Schmal.; genome formula DD) duplicated its chromosomes and became fertile, originating hexaploid wheat (*T. aestivum* L., genomes AABBDD), a species that includes the present common wheat cultivars.

The origin of the B genome is uncertain because no known diploid species ($2n = 14$) fits the cytological, morphological, and biochemical characteristics expected of the putative B genome donor. Possibly, such a species is either extinct or has not yet been discovered, but most experts agree that the B genome is probably a composite and that its chromosomes have been contributed by two or more diploid species. This composite might have occurred by hybridization between tetraploids containing a common A genome and different second genomes.

The cultivated wheats are part of an allopolyploid complex, which is a group of closely related diploid and allopolyploid species constituting the genera *Triticum* and *Aegilops*. Bowden (1959) and Morris and Sears (1967) proposed that these two genera be integrated into a single genus, *Triticum*. All of these species have one to three homoeologous genomes consisting of seven pairs of chromosomes each. More distant relatives of both wild and cultivated wheat, such as the genera *Agropyron*, *Secale* (rye), and *Hordeum* (barley), also have one or more genomes consisting of seven pairs of chromosomes each.

B. Aneuploids and Related Genetic Stocks

Chromosomes of the closely related diploid species that donated genomes to allohexaploid wheat seemingly underwent limited evolutionary changes with respect to their common ancestor, which means that considerable redundancy of genetic information exists in the allopolyploid. This redundancy and the existence of many linkage groups (21 pairs of chromosomes) make conventional genetic

analysis—the study of segregations of allelic variants—a difficult task. These same circumstances, however, made it possible to establish viable genetic stocks (aneuploids) that either lack or have extra doses of whole chromosomes or chromosome arms, compared to the normal (euploid) stocks. Because hexaploid plants are genetically redundant, the loss or the increase of a fraction of the genetic information, which would normally be lethal to diploid species, is only more or less deleterious. More chromosomally identified aneuploids have been obtained from wheat than from any other organism. The complete nuclear genetic complement of hexaploid wheat is, in fact, covered by different aneuploid series. This allows aneuploid analysis, a type of genetic analysis defined as the ordered, systematic silencing or enhancing of blocks of genetic information (chromosomes, chromosome arms, or chromosome segments) and the correlation of the genetic changes with concomitant phenotypic changes. Two types of aneuploids (monosomics and nullisomics) from the hexaploid (*Triticum aestivum*) cv. Chinese Spring have been available since Sears first obtained them in 1954. Many aneuploids were subsequently obtained from Chinese Spring wheat by Sears (1959, 1965, 1966, 1969, 1974, 1975), and many aneuploids and related stocks have been obtained from different cultivars by plant geneticists from many countries.

A monosomic is a stock that lacks one chromosome out of the 21 pairs that comprise the three genomes. A complete monosomic series is thus composed of 21 different types of plants, each lacking a different one of the 21 chromosomes. Most of the original Chinese Spring monosomics were obtained from haploids and asynaptics, but they occur spontaneously in most varieties, and a complete series can be recovered by cytological analysis of several hundred to a few thousand plants. Selfing of monosomics yields 20–25% normal plants (disomics), about 75% monosomics, and only 1–7% nullisomics, which are plants that lack one pair out of the 21 pairs of chromosomes. Nullisomics have a low frequency because on the female side, normal (21 chromosomes) and deficient (20 chromosomes) eggs segregate in a 1:3 ratio, whereas on the male side, normal pollen is strongly favored over deficient pollen. If enough progeny are grown, the complete series of nullisomics can be recovered by selfing the complete series of monosomics.

Assignment of genes that have a phenotypically detectable expression to particular chromosomes can be achieved by observing the disappearance or alteration of the expressed character in a particular nullisomic. In fact, nullisomics provided the first evidence of chromosome homoeology. Most nullisomics have reduced fertility and are much less vigorous than the euploid, so they have to be reisolated from the corresponding monosomics each time they are needed.

Trisomics and tetrasomics are stocks that have one and two extra doses, respectively, of one of the chromosomes, relative to the euploid. Trisomics segregate when selfed (1–10% tetrasomics, 45% trisomics, the remainder disomics). Tetrasomics are unstable, and most of them produce only about 80% tetrasomic progeny. Tetrasomics have also been useful for determining chromosome homoeologies. The establishment of compensated nullisomic-tetrasomic stocks was important in the final determination of chromosome homoeologies and in providing a versatile, stable tool for the aneuploid analysis of many different characters (Sears, 1965). Nullisomy for each chromosome of a

given genome was phenotypically compensated, to some extent, by two extra doses of one particular chromosome from each of the other two genomes. Thus, it was possible to place the 21 chromosomes of wheat in seven homoeologous groups containing three chromosomes each. Each homoeologous group included one chromosome from each genome. The 21 different chromosomes of wheat were then designated 1A-7A, 1B-7B, and 1D-7D (Sears, 1965).

In addition to the primary aneuploids, secondary aneuploids have been obtained in which the genetic dosage alteration occurs in the chromosomal arms. Telocentric chromosomes, which lack one arm, are available for each of the 42 arms (21 chromosomes) of hexaploid wheat in the form of monotelosomics and ditelosomics. Those that are nearly or completely sterile must be maintained as heterozygous individuals that carry one or two doses of the other arm from the same chromosome, and they must be recovered by selfing. The two telocentrics of a given chromosome are designated as long (L) or short (S) if their relative lengths are known, and as α or β if they are not known (Kimber and Sears, 1968).

Other genetic stocks of interest in the assignment of genes to chromosomes are those carrying either intraspecific (homologous) substitutions or alien (homoeologous) genetic material (chromosomes or chromosomal segments) from other species and genera whose genomes are homoeologous to those of wheat. Three types may be considered: alien additions, homologous or alien substitutions, and alien transfers.

Because of its hexaploid constitution, common wheat is highly genetically buffered and can tolerate the addition of alien chromosomes. Alien additions can be obtained by many procedures, which include interspecific crosses, synthesis of bridge allopolyploids, backcrosses to wheat, and selection of lines carrying alien chromosomes by checking for appropriate genetic markers. Monosomic and disomic additions have been obtained for chromosomes from different species of *Aegilops-Triticum*, such as *Ae. umbellulata* (*T. umbellulatum*), *Ae. comosa* (*T. comosum*), *Ae. ventricosa* (*T. ventricosum*), and from more distant relatives of wheat, such as rye (*Secale cereale*), and barley (*Hordeum vulgare*), *Agropyron elongatum*, and *Agropyron intermedium* (Dosba et al, 1979; Islam et al, 1975; Riley, 1960; Sears, 1975). Selfing of monosomic additions yields a lower frequency of disomic additions than with wheat chromosomes because the male gametes containing 21 chromosomes are usually strongly favored. Disomic additions are stable to varying degrees, with the stability depending on the chromosomes added.

Several intervarietal, interspecific, and intergeneric chromosome substitutions have been obtained. In the latter two types, increasing evidence suggests that the only lines that are completely successful are those in which the substitution is made with a homoeologous chromosome. Generally, a chromosome that substitutes successfully for one member of a homoeologous group will substitute well for the other two members of the group. Plants containing intervarietal substitutions are perfectly normal. Those with alien substitutions are generally stable, and many of them are reasonably vigorous and fertile.

Although wheat is a hexaploid, it functions as a diploid. Homoeologous chromosomes do not pair at meiosis, nor do they normally recombine, in spite of structural similarities that arise from their common origin. Such interactions are negated by diploidizing genes that prevent homoeologous pairing. Okamoto (1957), Riley and Chapman (1958b), and Sears and Okamoto (1958) found that a

gene (*Ph*) located in the long arm of chromosome 5B, prevents homoeologous pairing in wheat. Other genes affecting homoeologous pairing to different degrees were later identified by other investigators.

Through the use of any of several different procedures, manipulation of the *Ph* and similar genetic systems promotes recombination between alien and wheat chromosomes. Many interspecific and intergeneric transfers have been obtained in this way. In these transfers, segments from alien chromosomes replace homoeologous segments from wheat chromosomes. Exchange of segments between chromosomes can also be induced by ionizing radiation. Although radiation-induced exchanges tend to occur between homoeologous chromosomal segments, such exchanges may also occur between nonhomoeologous chromosomes. The frequency of radiation-induced exchanges is much lower than that of exchanges obtained by induced homoeologous pairing.

Despite the essential integrity of the homoeologous groups of chromosomes, which allows a high degree of genetic compensation when one chromosome is substituted by a homoeologous chromosome, some diversification of chromosome structure and genetic information has occurred by translocation (Riley et al, 1967) and by loss of redundant gene expression (Carbonero and García-Olmedo, 1969; García-Olmedo, 1968; García-Olmedo et al, 1978b). This diversification and the fact that tetraploid wheat is less genetically buffered than hexaploid wheat have made monosomics and other aneuploids of *T. turgidum* much more difficult to obtain (Joppa, 1973; Joppa et al, 1975, 1979; Longwell and Sears, 1963; Mochizuki, 1968; Noronha-Wagner and Mello-Sampayo, 1966; Sears, 1969).

C. Genetic Constitution of Wheat Endosperm

In addition to the genetic complexity arising from the allohexaploid nature of wheat, endosperm tissue is further complicated because of its triploid nature (AAABBDDDD). Of the three copies of each individual chromosome in an endosperm cell, two are contributed by the female gamete, and one is contributed by the male gamete. Thus, the endosperms of reciprocal hybrids between stocks with different alleles at a given locus (a^1 and a^2) will differ in genetic constitution ($a^1 a^1 a^2$ and $a^2 a^2 a^1$), depending on whether the maternal parent possessed a^1 or a^2 .

The implications of this special genetic constitution of wheat endosperm on the study of the genetic control of wheat endosperm proteins were first discussed by Favret and co-workers (Favret et al, 1970; Manghers et al, 1973; Solari and Favret, 1968) and were further elaborated by Wrigley (1976) and Salcedo et al (1978a).

D. Advantages and Limitations of Different Approaches to the Genetic Analysis of Wheat Endosperm Proteins

Conventional genetic analyses of segregating allelic variants and of linkage groups are not always feasible in an allohexaploid such as wheat. On the other hand, the rich variety of viable aneuploids and related stocks available for this species greatly facilitates genetic analyses down to the level of chromosomal

segments. Three main criteria can be applied to assign a chromosomal location to a given gene: concomitance of the absence of the phenotypic effect associated with the gene and the lack of a particular chromosome or chromosomal segment; quantitative variation of the level of expression of the phenotypic trait as a function of the dosage of a chromosome or chromosomal segment; and substitution or addition of a phenotypic trait in stocks in which a homologous or homoeologous substitution or addition has been done using a donor that has the variant phenotype.

Evidence corresponding to the first criterion can be obtained by analyzing a complete series of stocks such as nullisomics, nullisomic-tetrasomics or ditelosomics, in which the entire nuclear genetic information in wheat is systematically deleted one block at a time. Dosage effects can be investigated by analyzing monosomics, trisomics, tetrasomics, and nullitetrasomics. The complete series of compensated nullitetrasomics is particularly useful because they not only provide evidence of chromosomal location according to the first two criteria, but they usually have a more nearly balanced and normal development than other aneuploids. This makes it less likely that the nonspecific consequence of markedly abnormal plant development will occur. Evidence of the third criterion is obtained by the analysis of intraspecific homologous substitutions, interspecific or intergeneric homoeologous substitutions, monosomic and disomic addition lines, and alien transfers. This type of evidence is particularly useful in protein studies because it will often discriminate between structural and regulatory or modifier genes.

Monosomics can also be used for assigning genes to chromosomes in a different way (Sears, 1975). A dominant gene is located by crossing the stock that contains the dominant gene to each of the 21 monosomics, identifying the F_1 monosomics, and observing which F_2 population deviates strongly from a 3:1 ratio. Active recessive genes are only expressed when they are homozygous, and inactive recessive genes are not expressed even when homozygous. This procedure has seldom been used to study the inheritance of individual proteins (Kimber and Sears, 1980; Sears, 1975).

Telocentric chromosomes are also useful in gene-mapping (Kimber and Sears, 1980; Sears, 1966). By crossing the stock being analyzed to the two ditelosomics lacking arms of the chromosome that carries the gene under investigation, the arm on which the gene is located can be determined because recombinant telosomes carrying the gene will only be recovered from the appropriate ditelosomic. The frequency of recovery of such telosomes is a measure of the distance between the gene and the centromere.

Various situations must be considered for genes that encode wheat endosperm proteins and for genes that regulate or modify the expression of the structural genes. Not all genes of allohexaploid wheat are present in triplicate. One to three homoeologous loci may thus exist for a given biochemical system (protein). The products encoded by a duplicate or triplicate homoeologous set of genes may be identical or at least operatively indistinguishable by the available analytical methods. Alternatively, the products encoded by the different homoeogenes may be individually identifiable.

Expression of the structural genes may also be affected by regulator or modifier genes of which one to three copies may be present. The components of a duplicate or triplicate set of regulatory or modifier homoeogenes may be

equivalent in their action. That is, they would affect all the structural homoeogenes of a set to the same extent. Alternatively, they may diversify so that each member of the set acts differently or even on different structural genes. A pertinent question in genetic analysis is whether there is intraspecific, homologous variation or extraspecific, homoeologous variation for a given structural or regulatory homoeogene.

The assignment of the structural gene for a given protein to a particular chromosome, using aneuploids and related stocks, must be based on the observation that the protein is absent only in stocks lacking that chromosome but is present in stocks lacking any of the remaining chromosomes. Even in this most favorable case, however, alternative explanations, such as the possibility that triplicate genes encode the same gene product and that a single regulatory or modifier gene required for their expression, cannot be excluded. When the absence of more than one chromosome is associated with the absence of a protein, the assignment of the structural gene cannot be inferred directly, and additional information is required (García-Olmedo et al, 1978a).

Much of the ambiguity in chromosome assignment can be overcome when the particular protein genetic variant in question can be detected in an alien stock that carries, as an addition, a substitution, or a transfer, the chromosomal segment suspected of including the structural gene for the protein. This is also true when the said chromosomal segment is replaced by a homologous or homoeologous chromosome or chromosomal segment from a stock possessing a different variant of the protein, and the first protein variant is replaced by the second.

To ascertain the absence of a protein, the analytical procedure must be selective for that protein. Usually, one fractionation method alone (electrophoresis at various pHs, sodium dodecyl sulfate electrophoresis, and electrofocusing) will not attain enough resolution to positively identify most protein components of endosperm extracts. One-dimensional separations generally suffice only when a selective extraction procedure or staining method is available. Two-dimensional separations are more reliable. When more than one protein is present in a given fraction, such as an electrophoretic band, quantitative changes in that band that are associated with the lack of or the increased dosage of a chromosome can only indicate the possible location of structural genes and regulatory or modifier genes.

Complete characterization of protein fractions and individual proteins is necessary to properly interpret the genetic data, to overcome any lack of resolution due to analytical procedures, and to compare the findings of different research groups.

Conflicting assignments of structural genes for components of a given endosperm protein class have been reported by different research groups. Such discrepancies may have a number of causes, among which are differences in the extraction and fractionation procedures, differences in staining methods, and improper characterization of the individual protein components or of the genetic stocks studied.

III. CLASSES OF PROTEINS IN WHEAT ENDOSPERM

The classification of wheat endosperm proteins proposed by Osborne in 1907 was based primarily on solubility criteria and is still in use, despite its several

shortcomings. Kasarda et al (1976a) and Miflin and Shewry (1979) recently discussed the difficulties of applying Osborne's method.

Osborne (1924) proposed four classes of proteins: albumins, which are soluble in water; globulins, soluble in salt solutions; prolamins (gliadin in wheat), soluble in aqueous alcohols; and glutelins (glutenin in wheat), insoluble in any of the previous solvents. In practice, wheat endosperm proteins have often been classified according to their extractability by the different solvents of a sequential extraction. This type of classification can obviously lead to ambiguity because a given protein could be assigned to different classes, depending on the order in which the solvents are used. Other extraction conditions such as temperature, mechanical treatment during extraction, solvent/endosperm ratio, number of extractions with a given solvent, delipidation procedure, redistribution of lipids during extraction, and particle size of the milled endosperm could also affect the assignment.

Some proteins are incompletely extracted during a given step and thus also appear in a later fraction. This problem is particularly important in the two most abundant protein classes of wheat endosperm—gliadins and glutenins. Aqueous alcohols, which are fairly selective extractants for gliadins, do not seem to extract them quantitatively, so gliadin polypeptides are present as contaminants in subsequent glutenin fractions (Bietz and Wall, 1973, 1975; Brown and Flavell, 1981; Kasarda et al, 1976a; Miflin and Shewry, 1979). The extractability of gliadins is improved by using elevated temperatures and by adding a reducing agent such as 2-mercaptoethanol to the aqueous alcohol solvent (Miflin and Shewry, 1979). This type of treatment, however, alters the native covalent structure of the proteins under study because it breaks disulfide bridges and might lead to the inclusion of proteins in the gliadin fraction that are not soluble in aqueous alcohol when they are in their native state. Classification criteria based only on solubility (not on extractability), which have had a much more limited practical application in the classification of wheat endosperm proteins than the sequential extraction procedures, are also ambiguous because many proteins are soluble in more than one of the different solvent types. Typical wheat gliadins, for example, can be dissolved and extracted by water and neutral salt solutions (Doekes, 1968; Minetti et al, 1971, 1973; Pence et al, 1954; Shearer et al, 1975; Waines, 1973).

In studies of the genetic control of individual protein components from wheat endosperm, so many extraction and fractionation procedures have been used that it is often difficult to know how to apply Osborne's classification to a given group of proteins.

IV. LOCATION OF GENES ENCODING GLIADINS

Gliadins are the prolamins of wheat endosperm. Although gliadins seem to be more efficiently extracted with aqueous 2-propanol (Miflin and Shewry, 1979), 70% ethanol has been the solvent most frequently used to extract them from wheat flour, after extraction of albumins and globulins, or from salt-washed gluten (Kasarda et al, 1976a). Typical gliadins have been classified into α , β , γ , and ω components, based on fractionation by free boundary electrophoresis (Jones et al, 1959). This classification has since been extended by Woychick et al

(1961) to proteins separated by aluminum lactate (pH 3.2) starch gel electrophoresis (SGE). These proteins have apparent molecular weights of 30,000–80,000, judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Low molecular weight proteins in aqueous alcohol extracts have been considered to be albumin and globulin contaminants (Kasarda et al, 1976a). Many of these proteins, however, are hydrophobic and have peculiar solubility properties.

Gliadins characteristically contain large amounts of glutamine (≤ 30 residues per 100 amino acid residues) and proline (14–18 residues per 100 amino acid residues) and have a low content of lysine (< 1 residue per 100 total residues). They seem to be typical reserve proteins located in protein bodies, and their levels in the endosperm can be markedly altered by changing the nitrogen supply or by genetic selection (Kasarda et al, 1976a; Konzak, 1977; Mifflin and Shewry, 1979). The gliadins comprise a complex mixture of proteins that have similar amino acid compositions and properties. Considerable variation exists, however, among electrophoretic patterns of gliadins from different wheat varieties, and this variation has been valuable in cultivar identification. Nevertheless, the extensive homology of the prolamins allows considerable amino acid sequence information to be obtained from these proteins without the isolation of pure protein components. For example, Autran et al (1979) partially sequenced an unfractionated mixture of gliadins and found evidence of two major groups of prolamins in the *Triticum-Aegilops* species they examined; one had the α -type N-terminal sequence [Val-(Arg?)-Val-Pro-Val-Pro-Gln-Leu-], and the other had the γ -type sequence [Asn-(Met/Ile)-Gln-Val-(Val/Asp)-Pro-Gln-Gly-]. The α -type sequence was homologous with that found by Bietz et al (1977) for the α -, γ_1 -, and β_5 -gliadins from Ponca winter wheat, and the γ -type was similar to that of γ_2 - and γ_3 -gliadin from the same variety. Shewry et al (1980) recently reported that 23 of the first 27 amino acid residues from the N-terminus of a purified ω -gliadin component from *T. monococcum* are homologous with those of a C-hordein component from barley.

The first reports relating to the genetic control of electrophoretically separable gliadin components were published in 1967. Using aluminum lactate SGE to analyze gliadins from single kernels, Solari and Favret (1967) studied the inheritance of gliadin proteins in different genetic crosses and concluded that at least 11 loci, belonging to three linkage groups (chromosomes), were involved in controlling their synthesis. Furthermore, they observed gene dosage effects that caused the amounts of certain protein components to vary.

Boyd and Lee (1967) examined the gliadin electrophoretic patterns of 22 of the 42 possible ditelosomic lines of the wheat variety Chinese Spring and reported the disappearance of two slow-moving (ω -gliadin) bands when one arm of chromosome 1D was absent. In a later work, Boyd et al (1969) showed that when the entire D genome of the cv. Canthatch (Kerber, 1964) was removed, three slow-moving (ω -gliadin) bands and one intermediate (β -gliadin) band were missing. In both studies, gliadins were extracted with 2M urea and fractionated by aluminum lactate SGE. A more complete aneuploid analysis of gliadin inheritance was reported by Shepherd (1968), who used the same extraction and fractionation techniques as Boyd and Lee. Compensating nullitetrasonics (33 out of 42 possible) were investigated, as were 21 tetrasomics and 21 ditelosomics supplied by Sears (1954, 1966). The gliadin electrophoretic pattern was divided

into groups of bands designated J, K, L, and M, starting from the origin (equivalent to ω -, γ -, β -, and α -gliadins, respectively), and the bands in each group were numbered, starting from the band that moved the shortest distance into the gel. Nine of the 17 major gliadin bands were accounted for by the deletion of individual chromosomes: K₁ (1A); K₃, K₄, M₂ (1B); J₂, J₃ (1D); M₆, M₇ (6A); and M₅ (6D). Each of the remaining eight major protein bands was either controlled by more than one gene located in more than one chromosome (different proteins comigrating in electrophoresis or repeated genes encoding the same protein) or, less likely, genes controlling these proteins were all located in the right arm of chromosome 4A, which could not be tested at the time. By investigating correlations between chromosome dosages and relative band-staining intensities, Shepherd observed that five of the remaining eight bands were affected by dosage changes in chromosomes belonging to homoeologous groups 1 and 6: L₁ (1A, 1D), L₂ (1A, 1B), L₃ (1B, 1D), L₇ (1A), and L₄ (6B). The results obtained with ditelocentric stocks indicated that the genes responsible for gliadin pattern changes were located in the nonstandard arms of group 1 chromosomes (now designated 1A-short, 1B-short, and 1D-short) and in the standard arms of group 6 chromosomes (now designated 6A-short, 6B-short, and 6D-short). Shepherd also observed a quantitative effect associated with the group 2 chromosomes.

Using the same stocks, Mitrofanova, (1976) confirmed the observations of Shepherd. Shepherd (1968) also analyzed the prolamins found in lines involving the addition of chromosomes from rye onto a wheat background: *Secale cereale* cv. King II onto *T. aestivum* cv. Holdfast (Riley, 1960; Riley and Chapman, 1958a), and *S. cereale* cv. Imperial onto *T. aestivum* cv. Chinese Spring (Sears, 1975). From this investigation, Shepherd tentatively concluded that only one rye chromosome was involved in prolamins control, whereas in wheat, two chromosomes per genome affected gliadin synthesis. Shepherd (1973) confirmed that only one chromosome of rye (the chromosome E of Imperial rye), which was homoeologous with the group 1 chromosomes of wheat, controlled endosperm prolamins synthesis. He also showed that in *Aegilops umbellulata* two chromosomes (A and B), which are respectively homoeologous with groups 6 and 1 of wheat, were responsible for controlling the presence of prolamins.

Gliadins have also been investigated with intraspecific substitution lines. Substitution lines containing *T. aestivum* cv. Cheyenne chromosomes in Chinese Spring, obtained by Morris (Morris et al, 1966), were studied by Eastin et al (1967) and more recently by Kasarda et al (1976b). In the latter case, long gels were used for the aluminum lactate PAGE (pH 3.2) to improve resolution. Genetic control of 13 out of 25 detectable gliadin components in Cheyenne, and of 11 out of 22 gliadin components in Chinese Spring, was assigned to chromosomes of groups 1 and 6. In particular, the synthesis of A-gliadin, the aggregable α -gliadin subfraction described by Bernardin et al (1967), which may be a toxic factor involved in celiac disease, was controlled by the α -arm of chromosome 6A. A study of addition lines containing chromosomes from cv. Thatcher added to Chinese Spring (Solari and Favret, 1970) was less conclusive, because the identity of some of the stocks used was uncertain.

Sasek and Kösner (1977) studied plants resulting from the crossing of monosomic Chinese Spring (21 lines) with Kavkaz to determine the effect of the Kavkaz chromosomes on individual Kavkaz gliadin components. They assigned

control of some SGE (pH 3.1) bands to chromosomes 1B, 1D, 4A, and 6B. However, the proteins affected by chromosome 4A were not positively identified as typical gliadins. Genes for certain gliadins of the Odesskaya cultivar have been assigned to chromosome groups 1 and 6 by analyzing appropriate crosses of this cultivar with Chinese Spring aneuploids (Rybalko, 1975; Sozinov et al, 1978).

Wrigley (1970) fractionated wheat gliadins into more than 40 components by combined isoelectric focusing (IEF, pH 5-9) and aluminum lactate SGE (pH 3.2) (Fig. 2). This method indicated that some proteins that ran as single zones when separated by either method alone were, in fact, heterogeneous. The eight bands obtained by SGE of King II rye prolamins were similarly resolved into more than 20 components when subjected to the two-dimensional separation. Using this powerful separation method, Wrigley and Shepherd (1973) confirmed and extended the earlier findings of Shepherd (1968). Two-dimensional protein maps

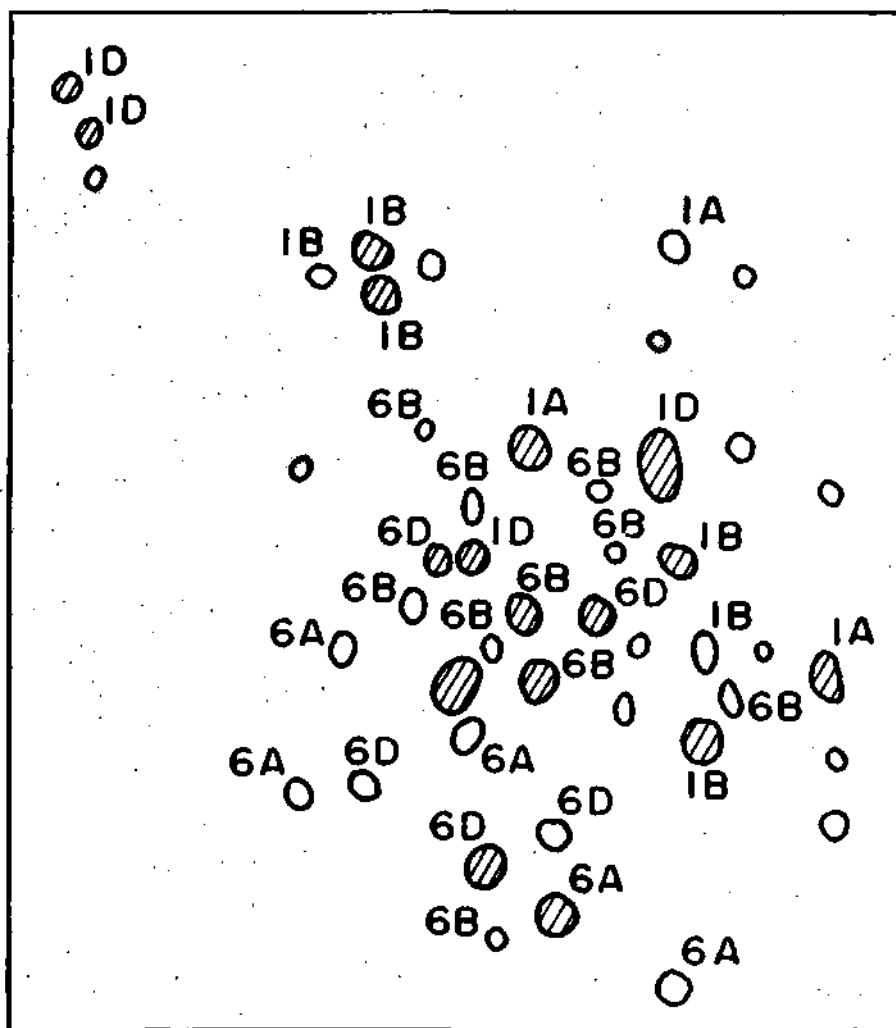


Figure 2. Two-dimensional protein map of the gliadins from variety Chinese Spring indicating the identity of chromosomes that control the synthesis of specific components. (Wrigley and Shepherd, 1973. Reproduced with permission of N.Y. Acad. Sci.).

of gliadins from compensating nullitetrasonics of Chinese Spring wheat, corresponding to homoeologous groups 1 and 6, were obtained, and 33 out of 44 components were assigned to specific chromosomes: 1A (3 components), 1B (6), 1D (4), 6A (5), 6B (10), and 6D (5). One major component could not be assigned, probably because more than one locus in more than one chromosome was involved in its genetic control. Control of the 10 remaining minor protein components was difficult to assign because detection of these proteins in a given stock depended on how much protein was loaded into the gel.

Brown et al (1979) and Brown and Flavell (1981) investigated the chromosomal locations of genes that control wheat endosperm proteins by a procedure that differed from that described by Wrigley and Shepherd (1973). They extracted the proteins with 2M urea, 0.5% SDS and 0.6% 2-mercaptoethanol and fractionated them by the method of O'Farrell (1975), IEF (pH 4.0-7.5), in the first dimension, and by SDS-PAGE in the second dimension (Fig. 3). Danno et al (1974) reported that this solvent extracted 95% of the

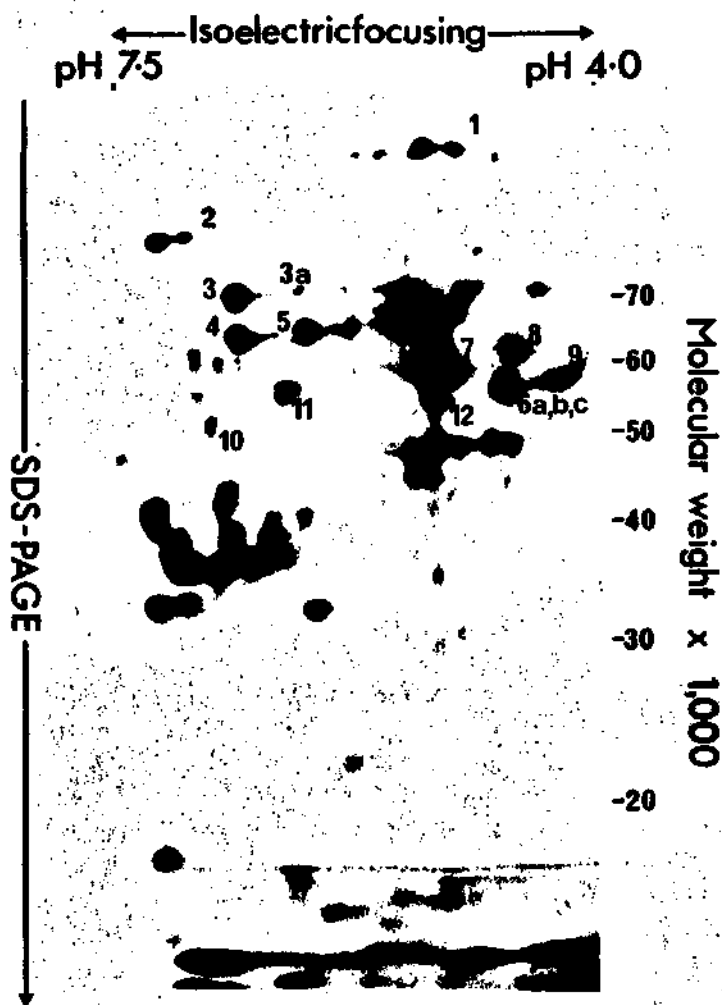


Figure 3. Two-dimensional electrophoresis protein pattern of the variety Chinese Spring. (Brown et al, 1979. Reproduced with permission of Genet. Soc. of United States).

endosperm proteins at room temperature within one hour. However, Brown et al (1979) used it at 4°C and extracted overnight. Because the solubility of SDS at 4°C is low and the critical micellar temperature is much higher (Helenius and Simons, 1975), 95% extraction may not have been achieved. Thirty-one components were resolved by the two-dimensional gel fractionation, 22 of which were classified as gliadins through the modified Osborne procedure of Chen and Bushuk (1970). Structural genes coding for 15 of these components were assigned to homoeologous chromosomes of groups 1 and 6, but control of seven of the proteins could not be assigned to any chromosome. An effect associated with the group 2 chromosomes was again found. The number of gliadin components affected by each of the chromosomes was 1A (0), 1B (5), 1D (5), 6A (2), 6B (1), and 6D (2). Using substitution lines, Brown et al (1981) investigated several hexaploid wheats and found that gliadin genes of these varieties are also located in chromosomes of groups 1 and 6.

The method of Wrigley and Shepherd (1973) resolved more gliadin components and assigned control of the synthesis of more of these proteins to given chromosomes than did the work of Brown et al (1979). The one exception was the 1D chromosome. Apparently, some of the ω -gliadins controlled by this chromosome are more acidic than the lower limit of the pH range (pH 5) used by Wrigley (1970), so they were not included in his protein map. Similarly, some of the gliadins have isoelectric points higher than the upper limit of the pH range (pH 7.5) used by Brown et al (1979). Proteins with very similar molecular weights and isoelectric points can be separated by SGE on the basis of their differential charges at acid pH, but they cannot be separated by SDS-PAGE. This may account for the greater proportion of the spots obtained by the method of O'Farrell (1975) remaining unassigned than those obtained by the method of Wrigley (1970).

A genetic analysis of intraspecific genetic variants of gliadin patterns, similar to that of Solari and Favret (1967), was done by Doekes (1973). Using one-dimensional gel electrophoresis, he found several different patterns among 101 selected lines derived from two crosses between *T. aestivum* cultivars. This established that the gliadin patterns were divisible into six or seven sections, the configurations of which were inherited unaltered (α -gliadins, one section; β -gliadins, two sections; γ -gliadins, one section; ω -gliadins, two to three sections).

The inheritance of gliadin components unique to the three wheat cultivars Cheyenne, Justin, and INIA 66R was investigated by Mecham et al (1978). They examined gliadins from appropriate crosses by using a two-dimensional fractionation technique—electrophoresis (pH 3.2) \times electrophoresis (pH 9.2) in polyacrylamide gels. They found that many of the gliadin bands segregated as if they were controlled by a single dominant gene. Linkage analysis provided evidence of codominant alleles and of closely linked genes (clusters of genes) that coded for gliadin components. One frequently overlooked finding of the work of Mecham et al (1978) is that, in their gliadin maps, several components migrated toward the cathode at pH 9.2. Because of their high isoelectric points, these components would not have been detected by Wrigley and Shepherd (1973) or by Brown et al (1979).

Convincing evidence suggests that genes encoding the typical gliadins are located in the short arms of chromosomes belonging to homoeologous groups 1 and 6, where they form closely linked clusters. However, certain gliadin

components with high isoelectric points might not have been included in the genetic studies.

V. LOCATION OF GENES ENCODING GLUTENIN SUBUNITS

The term "glutenin" was previously applied to different protein preparations that usually represented the least soluble fraction obtained from a sequential extraction of wheat endosperm. As the extraction procedures varied widely, so did the yield and composition of the glutenins obtained. A thorough review of the different glutenin preparation methods was published by Kasarda et al (1976a).

Orth and Bushuk (1974) were the first to attempt to study the genetic control of glutenin subunits. Glutenins were extracted from wheat grains with the solvent AUC (0.1M acetic acid, 3M urea, 0.01M hexadecyl trimethyl ammonium bromide) and were precipitated by adding ethanol to a concentration of 70% and adjusting the pH to 6.4 with 1M NaOH. The precipitated glutenins were redissolved in AUC, and SE-Sephadex C-50 was used to remove low-molecular-weight components (Orth and Bushuk, 1973a). Some doubts were raised about the selectivity of the Sephadex step (Kasarda et al, 1976a). The glutenins were then reduced with 2-mercaptoethanol and were fractionated by SDS-PAGE (pH 7.3). Through these procedures, the protein subunits coded by genes of the D genome were investigated by comparing the electrophoretic patterns of glutenins from three hexaploid (AABBDD) varieties with those of the corresponding tetraploid (AABB) varieties that had been obtained by genetic extraction of the D genome by Kaltsikes et al (1968). Three subunits with apparent molecular weights of 152,000, 112,000, and 45,000 were absent, and a fourth component of molecular weight 80,000 was greatly decreased in the lines that lacked the D genome (Orth and Bushuk, 1973b). These studies were then extended to the analysis of compensating nullitetrasonics and ditelosomics of Chinese Spring wheat to assign chromosomal locations to genes controlling glutenin subunit synthesis (Orth and Bushuk, 1974).

Four subunits (molecular weights 152,000, 112,000, 60,000, and 45,000) were present in euploid Chinese Spring and absent from nullitetrasonics that lacked chromosome 1D and from the tetraploid (durum) LD222. A 1D-1B substitution line of LD222 did contain the four subunits. Orth and Bushuk further observed that in lines that were tetrasomic for chromosomes 2B, 3B, and 6B, synthesis of glutenin subunits coded by either the A or B genomes was repressed. A different approach to glutenin preparation was used by Bietz et al (1975). A single-kernel analytical procedure was developed that was based on the sequential extraction studies of Bietz and Wall (1975). The sample (flour or ground kernel) was extracted twice with 0.04M NaCl and twice with 70% ethanol. The residue was then suspended in 0.7% acetic acid, ethanol was added to a concentration of 70%, and the pH was brought to 6.6-8.0 with 2N NaOH. After centrifugation, glutenin was extracted from the pellet with 0.125M tris-borate, pH 8.9, 1% 2-mercaptoethanol, and 0.1% SDS (plus 4-5 mg/kernel of dry SDS) at 37°C for 16 hours. SDS-PAGE was done in the same buffer (Fig. 4). Protein extraction was virtually complete with this procedure. Analysis of nullitetrasonics and ditelosomics showed that the presence of two glutenin subunits (molecular weights 104,000 and 93,000) was associated with the long arm of chromosome 1B. Genes controlling two other proteins (molecular weights 133,000 and 86,000)

were similarly assigned to the long arm of chromosome 1D (Fig. 4). A fifth protein, which at the time was classified as a glutenin but was later shown to be a high-molecular-weight globulin (Brown and Flavell, 1981), was controlled by chromosome 4D (long arm). These findings were confirmed by studying D-genome addition and substitution lines of durum wheat (Joppa et al, 1975, 1979) and Cheyenne-Chinese Spring substitution lines (Morris et al, 1966).

The results of Bietz et al (1975) differ markedly from those of Orth and Bushuk (1973a, 1973b; 1974) both in the chromosomal location of genes encoding glutenins and in the repression effects of tetrasomics. Differences in experimental procedures or possible errors in the identification of genetic stocks may account for the discrepancies. The AUC solvent is a less efficient extractant than the SDS-2-mercaptoethanol buffer used by Bietz et al (1975), and glutenins prepared by other methods lack or have a low proportion of some glutenin subunits (Bietz and Wall, 1975). Furthermore, SDS-PAGE gels used at pH 7.3 seemed to achieve less resolution and stained more poorly than those run at pH 8.9 (Bietz et

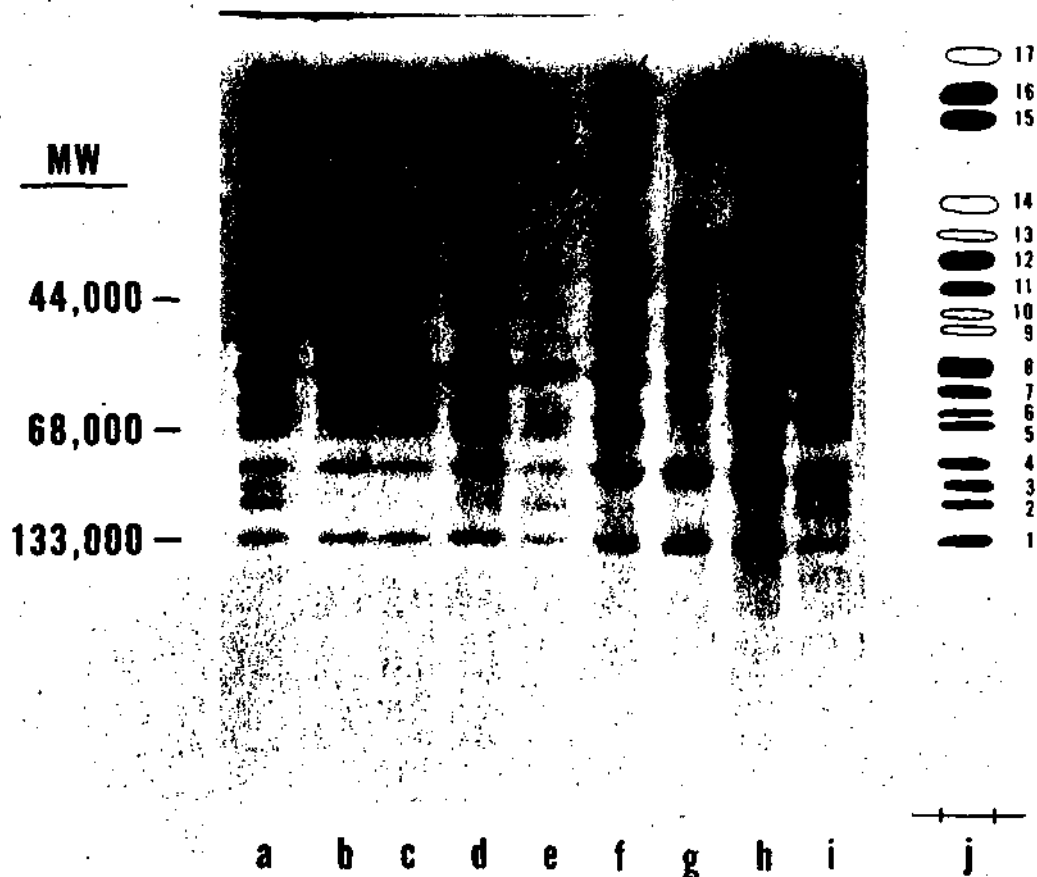


Figure 4. Analysis of glutenins by sodium dodecyl sulfate electrophoresis in polyacrylamide gels from single kernels of euploid and of 1B aneuploids of Chinese Spring: a, Chinese Spring; b, N1BT1A; c, N1BT1D; d, ditelo 1B₁; e, ditelo 1B₂; f, acetic acid extract of N1BT1D; g, HgCl₂ extract of N1BT1D flour after acetic acid extraction; h, 2-mercaptoethanol extract of N1BT1D flour after acetic acid and HgCl₂ extractions; i, Chinese Spring; and j, subunits in Chinese Spring (Bietz et al. 1975).

al, 1975).

Control of several low-molecular-weight components from the glutenin preparation of Bietz et al (1975) could not be assigned to particular chromosomes, probably because some of the electrophoretic bands obtained by SDS-PAGE contained more than one protein. Most gliadins are not completely extracted from flour unless 2-mercaptoethanol is used, so some of the bands probably represent true gliadins (Brown and Flavell, 1981; Mifflin and Shewry, 1979).

Brown et al (1979) and Brown and Flavel (1981) used the two-dimensional method of O'Farrell (1975) to fractionate glutenin obtained by two procedures—the modified Osborne procedure of Chen and Bushuk (1970), and a gel filtration method (Huebner and Wall, 1976; Payne and Corfield, 1979). They confirmed the presence of gliadins in their glutenin preparations and found two components (molecular weights 125,000 and 88,000) that were present exclusively in the glutenin fraction (Fig. 3). The presence of these components was controlled by chromosome 1D (long arm), and they are probably equivalent to the molecular weight 133,000 and 86,000 subunits of Bietz et al (1975). Each of these components results in more than one spot on the two-dimensional protein map, perhaps because of chemical modification during fractionation (carbamylation) or because they may represent true genetic heterogeneity (components 1 and 2 in Fig. 3). The two components with genes that Bietz et al (1975) assigned to chromosome 1B (long arm) seem to have isoelectric points outside the range normally used by Brown et al (1979) for their IEF step and therefore would not be consistently observed in the two-dimensional map. Brown et al (1979) also observed two additional protein components of molecular weights 50,000 and 53,000 that are controlled by chromosomes 1A (short arm) and 1D (short arm) (components 10 and 11 of Fig. 3). They do not seem to be glutenins because they are present in the Osborne glutenin, but they are not present in glutenin prepared according to Payne and Corfield (1979).

Lawrence and Shepherd (1980) reported on the genetic variation of glutenin subunits with apparent molecular weights of 80,000–140,000. The number of bands in each of 98 cultivars ranged from three to five, and at least 34 different band patterns were observed. In these patterns, some bands or band combinations were mutually exclusive and could be assigned to three groups, encoded respectively by genes located in chromosomes 1A, 1B, and 1D. Payne et al (1980) also found three to five glutenin subunits in each of seven varieties studied. Using intervarietal substitution lines, they concluded that two subunits were under the control of the 1D chromosome, 1 or 2 were controlled by chromosome 1B, and 0 or 1 by chromosome 1A. Brown et al (1981) investigated substitution lines from eight varieties and again found that the glutenin subunits were encoded by genes located in chromosomes 1A, 1B, and 1D.

Lawrence and Shepherd (1981) studied the chromosomal locations of genes encoding typical prolamins and high-molecular-weight glutenins in plant species related to wheat. Their studies indicate that chromosome 5 of barley, chromosome 1R of rye, chromosome 1 of *Ag. elongatum* and possibly chromosome 1C^u of *Ae. umbellulata* are similar to chromosomes 1A, 1B, and 1D of hexaploid wheat in that they carry genes controlling prolamins on their short arms and genes controlling glutenins on their long arms. These findings support the idea that all of these chromosomes are derived from a common ancestral

chromosome and that they have maintained their integrity.

Miflin et al (1980) recently proposed that if a polypeptide is completely soluble in an alcohol only when a reducing agent is present, it may still be classified as a prolamin. They also presented evidence that the high-molecular-weight glutenins described in this section must be considered as prolamins under such a definition. Nevertheless, these proteins are clearly different from the α -, β -, γ -, and ω -gliadins.

VI. GENETIC CONTROL OF PUROTHIONINS

Purothionins are low-molecular-weight, high-cystine, very basic proteins. In wheat flour, they form lipid-protein complexes that can be extracted with petroleum ether (Balls et al, 1942). They can also be extracted with salt solutions (Nimmo et al, 1968) or with dilute acid (Fernandez de Caleyá et al, 1976). Purothionins differ markedly from most flour proteins because they contain large amounts (about 20%) of cystine and of basic amino acids (about 20% lysine + arginine), but only small amounts of glutamine and proline (Redman and Fisher, 1968). SGE at pH 3.2 separates bread wheat purothionins into two components called the α - and β -forms (Redman and Fisher, 1968). The α -purothionin fraction from hexaploid wheat was separated into two fractions, α_1 - and α_2 - by ion exchange chromatography (Jones and Mak, 1977). The amino acid sequences of the three purothionin forms were determined (Hase et al, 1978; Jones and Mak, 1977; Mak, 1975; Mak and Jones, 1976a, 1976b; Ohtani et al, 1975, 1977). All three have very similar amino acid sequences, and each consists of 45 amino acid residues (Fig. 5).

Purothionins are toxic to bacteria, yeasts (Fernandez de Caleyá et al, 1972; Hernandez-Lucas et al, 1974; Stuart and Harris, 1942), vertebrates (Coulson et al, 1942), and insect larvae (Kramer et al, 1979). The physiological function of the purothionins is unknown, but they seem to be associated with the endoplasmic reticulum and are potent inhibitors of an in vitro wheat translation system (Carbonero et al, 1980; García-Olmedo et al, 1981). They have been reported to specifically kill cultured mammalian cells during the DNA synthesis phase of growth (Nakanishi et al, 1979), to affect membrane permeability, and to inhibit protein, RNA, and DNA synthesis (Carrasco et al, 1981). They also inhibit λ phage transcription in *Escherichia coli* (Ishii and Imamoto, cited in Ozaki et al, 1980).

Carbonero and García-Olmedo (1969) extracted purothionins from 22 *Aegilops* and *Triticum* species and used electrophoresis to separate them into α - (α^1 -, α^2 -, or both) and β -forms. They found that the diploid wheat species *Triticum monococcum* (genome A, one of three putative bread wheat progenitors) contained only β -purothionin. *Ae. squarrosa*, the proposed donor of the D genome to bread wheat, contained only α -purothionin, as did *Ae. speltoides*, which at that time was thought to contain the B genome of bread wheat. Of the other diploid *Aegilops* species checked, five contained α -purothionin, and two had the β form. None of the diploid species investigated possessed both α - and β -purothionins. Both durum wheat (*T. turgidum*, AB genomes) and bread wheat (ABD genomes) contained a mixture of α - and β -purothionins. This work thus indicated that one of the A genome chromosomes probably contains the gene coding for β -purothionin, and that the

B and D genomes carry genes for α -purothionin(s). This was later confirmed by García-Olmedo et al (1976) and Fernandez de Caleyá et al (1976). Using densitometry, they quantitated the amounts of the α and β forms present after electrophoretic fractionation of petroleum-ether extracted purothionins and found α : β ratios of 2:1 and 1:1 in *T. aestivum* and *T. turgidum*, respectively. Using euploid and nullitetrasonic lines of Chinese Spring (hexaploid) wheat, they also demonstrated that the only chromosomes that affected the presence of purothionins in the grains belonged to homoeologous groups 1 and 5. Whenever the 1A chromosome was removed (nulli 1A-tetra 1B, nulli 1A-tetra 1D) β -purothionin was missing. Absence of chromosomes 1B or 1D and double dosage of chromosome 1A (nulli 1B-tetra 1A, nulli 1D-tetra 1A) caused a reduction of α -purothionin and an increase in β -purothionin, whereas nulli 1B-tetra 1D and nulli 1D-tetra 1B lines contained purothionin complements equivalent to that of the euploid wheat. This was consistent with the location of the structural genes for α -purothionin(s) on chromosomes 1B and 1D and the one coding for β -purothionin on chromosome 1A. Ditelosomic lines 1AL, 1BL, and 1DL gave electrophoretic patterns identical to those of the euploid, indicating that the purothionin structural genes, designated *Pur-A1*, *Pur-B1*, and *Pur-D1*, are located in the long arms of their respective homoeologous group 1 chromosomes. Essentially the same results were obtained for group 1 aneuploids when purothionins were extracted with dilute H₂SO₄. Similar experiments showed that the absence of a gene or genes located in the short arm of chromosome 5D markedly decreased the yield of purothionins in the petroleum-ether extract but not in the dilute acid extract (Fernandez de Caleyá et al, 1976). This observation allowed the authors to discount a regulatory role for chromosome 5D in purothionin synthesis and further indicated that the 5D chromosome was possibly involved in the synthesis of lipids required for the solubility of the lipo-purothionin complex in petroleum ether (Fernandez de Caleyá et al, 1976; García-Olmedo et al, 1976). This was confirmed by reconstitution experiments involving the purothionin-lipid complexes. These allowed Hernandez-Lucas et al (1977a) to identify digalactosyldiglyceride (DGDG) as a lipid required for solubility of the lipoprotein complex in petroleum ether.

Genetic analysis showed that a gene(s) in the short arm of chromosome 5D did indeed affect DGDG levels as postulated (Carbonero et al, 1979; Hernandez-Lucas et al, 1977b). These results also agreed with the earlier finding that the yields of petroleum-ether extractable purothionins were lower in tetraploid than in hexaploid wheats (García-Olmedo et al, 1968). Because Pomeranz and

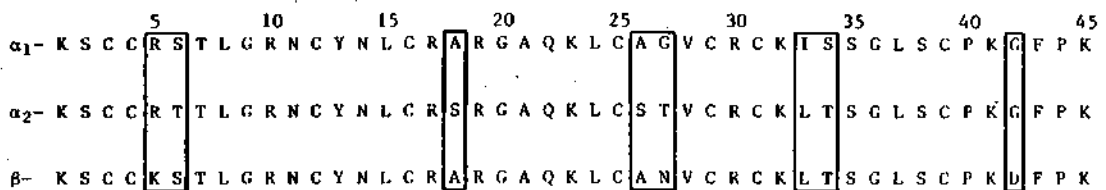


Figure 5. Amino acid sequences of the purothionins. Single letter amino acid notations are from Hunt et al (1976). Boxed areas indicate positions where the proteins differ in amino acid sequence. (Data from Mak, 1975.)

co-workers (Pomeranz, 1971) showed that DGDG may affect bread volume, the results also suggest that the differences in breadmaking quality between durum and common wheats may be partially related to other chemical moieties besides storage proteins that are controlled by the D genome.

Fernandez de Caleyá et al (1976) purified α - and β -purothionin forms from *T. turgidum* cvs. Senatore Capelli and Bidi 17 and from *T. aestivum* cv. Aragon 03 and β -purothionin from *T. monococcum* (AP line) and analyzed their amino acid compositions. By comparing the amino acid composition of α -purothionin from *T. turgidum* (containing only one α form) with that of the α fraction from *T. aestivum* (with two α forms, designated α_{II} and α_{I1}), they calculated that the sequences of the two α forms probably differed in at least four amino acid positions. These observations were in agreement with their genetic model.

A more precise and thorough characterization of the genetic variation among the purothionin forms was conducted using protein sequencing studies. Pure samples of purothionins were extracted from *T. monococcum*, *T. turgidum*, and *T. aestivum* and were sequenced (Jones and Mak, 1977; Jones et al;¹ Mak, 1975; Mak and Jones, 1976a, 1976b). *T. monococcum* (A genome) contains only one purothionin specie, and this protein has the same amino acid sequence as the β -purothionin of *T. aestivum*. Durum wheat (AB genomes) yields two forms of purothionin. The two proteins are present in essentially equal amounts and have the same amino acid sequences as the α_1 - and β -purothionins from *T. aestivum*. *T. aestivum*, which has three genomes (ABD), has three purothionins (α_1 -, α_2 -, and β -) that are highly homologous, differing from each other in five or six positions, depending on which forms are being compared.

Very strong homologies are found in the amino acid sequences of the various purothionins isolated from the three wheat species, and the three forms probably evolved from a common ancestor. Sufficient material has not yet been isolated from *Ae. squarrosa* to allow sequencing of its "purothionin," but its amino acid sequence will probably be identical to that of α_2 -purothionin from bread wheat because *Ae. squarrosa* is the donor of the D genome to *T. aestivum*. The same should be true of the B genome donor to the durum and bread wheats, so that whatever species donated the B genome to the original tetraploid wheat should contain a protein very similar or identical to α_1 -purothionin.

The genes coding for the purothionins appear relatively stable because no detectable mutational events occurred in the β -purothionin genes of either *T. monococcum* or of the polyploid wheats after the A and B genomes were combined to form the original tetraploid wheat. Likewise, the α_1 -purothionin gene was not altered in either durum or bread wheat lines after the D genome was added to tetraploid wheat.

The evolution of the purothionins in wheats is then straightforward (Fig. 6). A primitive plant acquired a gene that coded for a protein similar to purothionins, presumably before the monocotyledonous and dicotyledonous plant lines separated, because several dicotyledonous mistletoe species (Samuelsson, 1973; Samuelsson and Pettersson, 1971) contain proteins (viscotoxins) that are remarkably homologous with purothionins. The gene was carried down to an

¹B. L. Jones, A. S. Mak, D. B. Cooper, and G. L. Lookhart. The amino acid sequences of purothionins from durum wheat and from *Triticum monococcum*. Unpublished data.

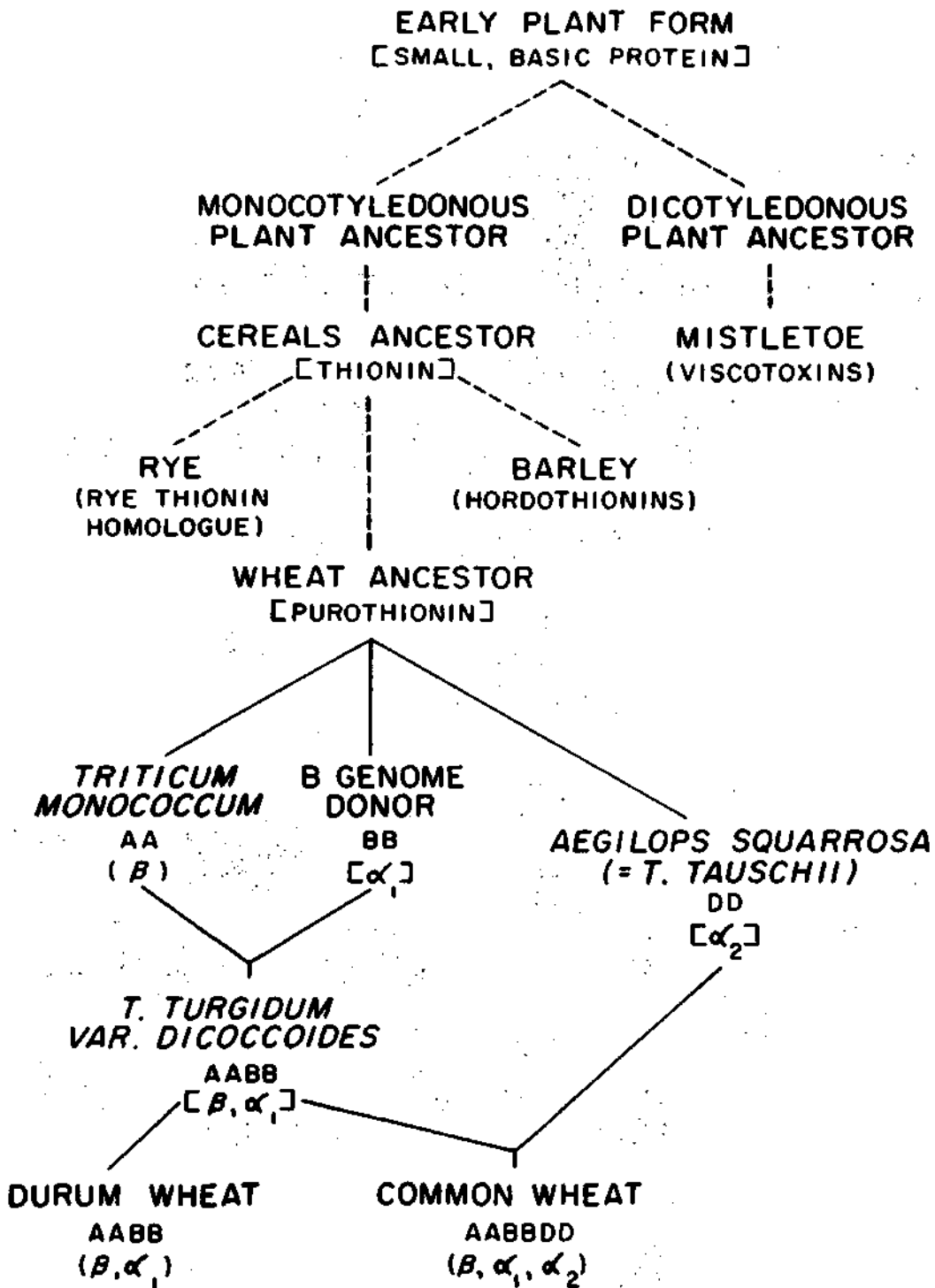


Figure 6. Proposed inheritance of purothionins and related proteins. Species are listed with their demonstrated or postulated protein complements. () = demonstrated (sequenced or partially sequenced) proteins; [] = postulated proteins. Genomes of the *Triticum* species are listed.

ancestor common to the different Triticineae species. This ancestral plant form apparently evolved into several species, including the three bread wheat progenitor species *Triticum boeoticum* (syn. *T. monococcum*), *Ae. squarrosa*, and the still undetermined B genome donor. During this period, enough mutations occurred to appear as five and six amino acid differences in the purothionins of the different diploid species. Some time after the A and B genome donor species hybridized, the D genome was added by a second hybridization. Before this event, no amino acid changes occurred, at least in the A genome product (β -purothionin).

Redman and Fisher (1969) found a purothionin homologue in barley, which they named hordothionin. Mak (1975) isolated two hordothionin fractions from barley. One fraction contained one pure protein, and the other fraction contained two proteins that were so similar they could not be separated. Both fractions were subjected to amino acid sequence analysis, and at least three different, highly homologous proteins were present. Ozaki et al (1980) sequenced a hordothionin that they isolated from a commercial barley flour. Their hordothionin is apparently the same as the β -hordothionin of Mak, even though the reported amino acid sequences differ slightly. All of the hordothionins are highly homologous with purothionins, and at least one has cystine disulfide bridges at exactly the same positions as they are found in purothionins (Hase et al, 1978; Ozaki et al, 1980).

Hernandez-Lucas et al (1978) reported a purothionin homologue in rye. The genetic control of the rye thionin was studied in addition, substitution, and translocation lines in which rye chromosomes or chromosome segments were inserted into wheat cultivars (Sanchez-Monge et al, 1979). The thionin from rye migrates on SGE like β -purothionin, and its structural gene is located on the long arm of chromosome 1R. This location is homoeologous to those of the wheat purothionin genes, which are located on the long arms of wheat group 1 chromosomes.

VII. GENETICS OF LOW-MOLECULAR-WEIGHT HYDROPHOBIC PROTEINS

The extractability of some wheat endosperm proteins by chloroform-methanol mixtures was first reported by Meredith et al (1960). It was subsequently shown that the chloroform-methanol extracted proteins consisted of gliadins and what was considered to be albuminlike or globulinlike contaminants (Meredith, 1965a, 1965b, 1965c). Later work showed that the protein mixture extracted with chloroform-methanol (2:1, v/v) can be separated into two fractions by gel filtration on Sephadex G-100. One peak eluted as if it contained proteins of 30,000–90,000 molecular weight, whereas the second contained material of molecular weight less than 25,000 (Rodriguez-Loperena et al, 1975a). The first fraction contained a mixture of α -, β -, γ -, and ω -gliadins, as shown by aluminum lactate SGE. The lower molecular weight fraction was composed almost entirely of two clearly defined groups of hydrophobic proteins: the CM proteins (García-Olmedo and García-Faure, 1969; García-Olmedo and Carbonero, 1970; Redman and Ewart, 1973; Rodriguez-Loperena et al, 1975a; Salcedo et al, 1978a), and the low-molecular-weight gliadins (LMWG) (Prada et

al, 1982; Salcedo et al, 1979, 1980a).

The CM proteins migrate ahead of the LMWG on SGE at pH 3.2 and stain under conditions in which typical gliadins do not (Aragoncillo et al, 1975a). Five components, designated CM1, CM2, CM3, 16, and 17, were found in *T. aestivum* cultivars (Fig. 7). These proteins are apparently genetically invariant in hexaploid wheats (García-Olmedo and García-Faure, 1969; Rodríguez-Loperena et al, 1975a). In tetraploid wheat, however, an infrequent allelic variant of CM3 exists, designated CM3' (Rodríguez-Loperena et al, 1975a; Salcedo et al, 1978b). Proteins CM1, CM2, CM3, 16, and 17 from *T. aestivum*, and CM2, CM3, CM3', and 16 from *T. turgidum* have been purified and partially characterized (García-Olmedo and Carbonero, 1970; Redman and Ewart, 1973; Salcedo et al, 1978a). Their molecular weights are 12,000–13,000, and the outstanding feature of their amino acid compositions is that they contain a high

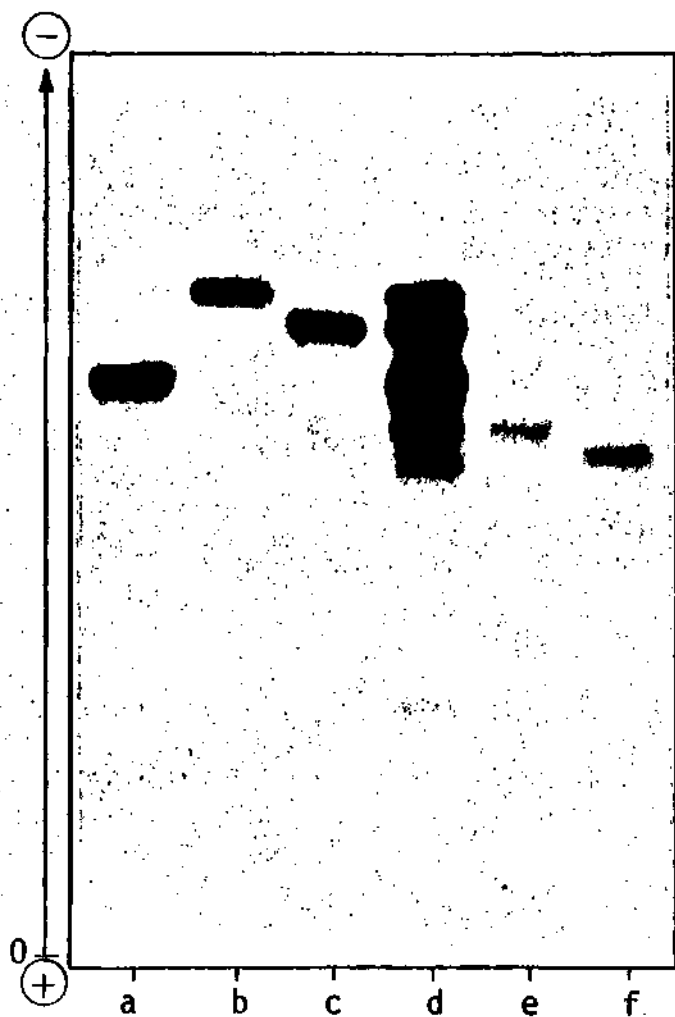


Figure 7. Starch gel electrophoresis (0.1 M aluminum lactate buffer, 3 M urea, pH 3.2; 2.5 hour run at 20 V/cm and 5°C; gels were stained with 0.5% Nigrosine in MeOH-H₂O-HOAc, 5:5:1, for 16 hours) of the following samples: a, purified CM3; b, purified CM1; c, purified CM2; d, CHCl₃-MeOH 2:1 extract from *T. aestivum* cv. Candéal; e, purified 16; f, purified 17. (Salcedo et al, 1978a. Reproduced with permission from *Phytochemistry*).

proportion of hydrophobic amino acids (49–59% of the total amino acid residues, excluding cysteine and tryptophan). They also contain lower glutamine and proline, and higher lysine concentrations than do typical gliadins. The high proportion of hydrophobic residues in these proteins probably explains their solubility in organic solvents. The CM proteins can also be extracted efficiently with 70% ethanol but not with water, although they can be made water-soluble by dialysis against an acid buffer (pH 3.2) containing 3M urea, without losing their solubility in organic solvents (Rodríguez-Loperena et al, 1975a).

García-Olmedo and García-Faure (1969) reported that flours from tetraploid wheats apparently lacked protein CM1. Based on this finding, they proposed a method for detecting flour from common (hexaploid) wheat in pasta products. The assignment of the gene coding for CM1 to the D genome was later confirmed by analysis of tetraploid wheats derived by extraction of the D genome from hexaploid wheat (García-Olmedo and Carbonero, 1970). Genes encoding CM1 and CM2 were assigned to chromosomes 7D and 7B, respectively, by analysis of their presence in monosomic and ditelosomic lines of Chinese Spring wheat (García-Olmedo and Carbonero, 1970). Rodríguez-Loperena et al (1975a) used a two-dimensional method based on that of Wrigley (1970) to fractionate the CM proteins. By joint mapping and sequential extraction, they showed that CM proteins, especially 16 and 17, were extracted more efficiently by 70% ethanol than they were by chloroform-methanol. Some of the CM proteins separated to give two spots on the two-dimensional map, probably because of carbamylation. Using the same technique, Aragoncillo et al (1975b) investigated the lines of compensating nullitetrasonics and ditelosomics of Chinese Spring wheat. They analyzed the components of the CM protein fraction that were soluble in 70% ethanol and had molecular weights of less than 25,000. They found that proteins CM1 and CM2 were coded by the short arms of chromosomes 7D and 7B, respectively, which agreed with the previous finding of García-Olmedo and Carbonero (1970). A minor component, protein 11, was also controlled by the short arm of chromosome 7D. The genes for proteins CM3 and 16 were located in the β arm of chromosome 4A, and the gene for protein 17 was in chromosome 4D. The amino acid compositions, molecular weights, solubilities, and genetic relationships indicate that homoeologous relationships exist between CM1 and CM2, and between 16 and 17 (Salcedo et al, 1978a). Waines (1973) previously conducted a similar study in which proteins extracted with 70% ethanol were fractionated by a one-dimensional electrophoretic method that did not resolve many of the individual components. The results obtained by Aragoncillo et al (1975b) and Waines may be correlated as follows. CM3 may form part of the 83 mm band of Waines (1973); proteins 16 and 17 would be part of the bands at 69 and 65 mm, respectively; CM1 is probably identical to the band at 105 mm; CM2 would be included in Waines' wide band covering the area between 90 and 100 mm. This implies that CM proteins were probably included among those used by Johnson and his co-workers in their taxonomic and phylogenetic studies (Hall et al, 1966; Johnson, 1972; Johnson and Hall, 1965; Waines, 1969).

Genes that encode CM proteins and are located in the short arm of chromosome 7D were further mapped by Rodríguez-Loperena et al (1975b), using the 7D/7Ag wheat-*Agropyron* homoeologous chromosome transfer lines synthesized by Sears (1972, 1973). In *Agrus* wheat, which is a 7D/7Ag substitution line of *Agropyron elongatum* into wheat (Quinn and Driscoll, 1967),

CM proteins normally found in *Agropyron* replaced those normally encoded by the 7D chromosome of wheat. Chromosomal transfer lines, in which terminal segments of the *Agropyron* chromosome 7Ag had replaced homoeologous wheat 7D chromosome segments, contained unaltered wheat CM proteins, whereas other transfer lines that included the centromere region of chromosome 7Ag had the *Agropyron* CM proteins and not the wheat CM proteins. It was thus concluded that genes encoding CM proteins must be proximal to the centromere in the short arms of chromosomes 7D and 7Ag of wheat and *Agropyron*, respectively. These results were also significant because they demonstrated that intergeneric homoeology exists at the level of chromosomal segments.

The second group of hydrophobic proteins, described by Salcedo et al (1979), are the LMWG. These proteins have molecular weights of 16,000–19,000, are soluble in 70% ethanol, and have electrophoretic mobilities in SGE (pH 3.2) similar to those of α -, β -, and γ -gliadins (Fig. 8). Purified components of the group have amino acid compositions that fall within the prolamins ranges suggested by Mifflin and Shewry (1979): >20% glutamine (23–27% in LMWG), >10% proline (9.1–11.4%), and <2% lysine (0.0–0.3%). SGE (pH 3.2) revealed that a total of 6–8 LMWG components were in *T. aestivum*, and 4–6 were in *T. turgidum*. A two-dimensional electrophoretic separation (pH 9 \times pH 3.2) yielded 10 LMWG components, all of which had high isoelectric points, as evidenced by their migration toward the cathode in the first dimension (pH 9.0). Several of these proteins have been purified and partially characterized (Prada et al, 1982).

Five major LMWG components were detected in crude, single-kernel extracts of Chinese Spring stocks by two-dimensional electrophoresis, and the variability and genetic control of these proteins were investigated (Salcedo et al, 1980a). In contrast with CM proteins, intraspecific variants of LMWG were found. However, their variability seems to be lower than that of the typical gliadins. After analyzing compensating nullitetrasonic lines, a ditelosomic 4A α line, and Blau-Korn (a 4A/5R wheat-rye chromosome substitution line), the genes controlling the synthesis of two proteins designated LMWG-1 and LMWG-6 were assigned to the 4B chromosome. The genes controlling proteins LMWG-2, LMWG-3, and LMWG-4 were tentatively assigned to chromosome group 7. The amount of LMWG-2 present was greatly decreased in nulli 7A-tetra 7B and in nulli 7A-tetra 7D lines, and the protein was apparently absent from the ditelosomic 7A (long arm) line. At the same time, LMWG-2 concentration was not affected by the absence of other chromosomes of group 7. LMWG-3 concentration was markedly decreased in stocks nullisomic for chromosome 7D but was not affected by the absence of either chromosome 7A or 7B. Protein LMWG-4 appears to be absent from all stocks nullisomic for chromosome 7D.

Notably, both CM proteins and LMWG are controlled by chromosomes of groups 4 and 7 and not by chromosomes of groups 1, 2, and 6, where the genes controlling typical gliadins are located. Salcedo et al (1980b) and Aragoncillo et al (1981) investigated two groups of proteins found in barley endosperm that correspond to the CM proteins and LMWG of wheat.

VIII. ALPHA-AMYLASE INHIBITORS AND RELATED PROTEINS

In 1943, protein inhibitors of α -amylase were discovered to exist in the endosperm of wheat grains (Kneen and Sandstedt, 1943). Reviews covering the

molecular properties, the biology (including genetics), and the possible nutritional significance of wheat α -amylase inhibitors were published by Marshall (1975) and by Buonocore et al (1977). The inhibitors in wheat are known to suppress α -amylase enzyme activity from many sources (Buonocore et al, 1977; Silano et al, 1975), but no one has yet reported finding any inhibitor in wheat grains that affects α -amylase from unmalted wheat. Proteins have, however, been extracted from both malted and unmalted wheats that can inhibit α -amylase enzyme extracted from malted wheat (Warchalewski, 1977a, 1977b).

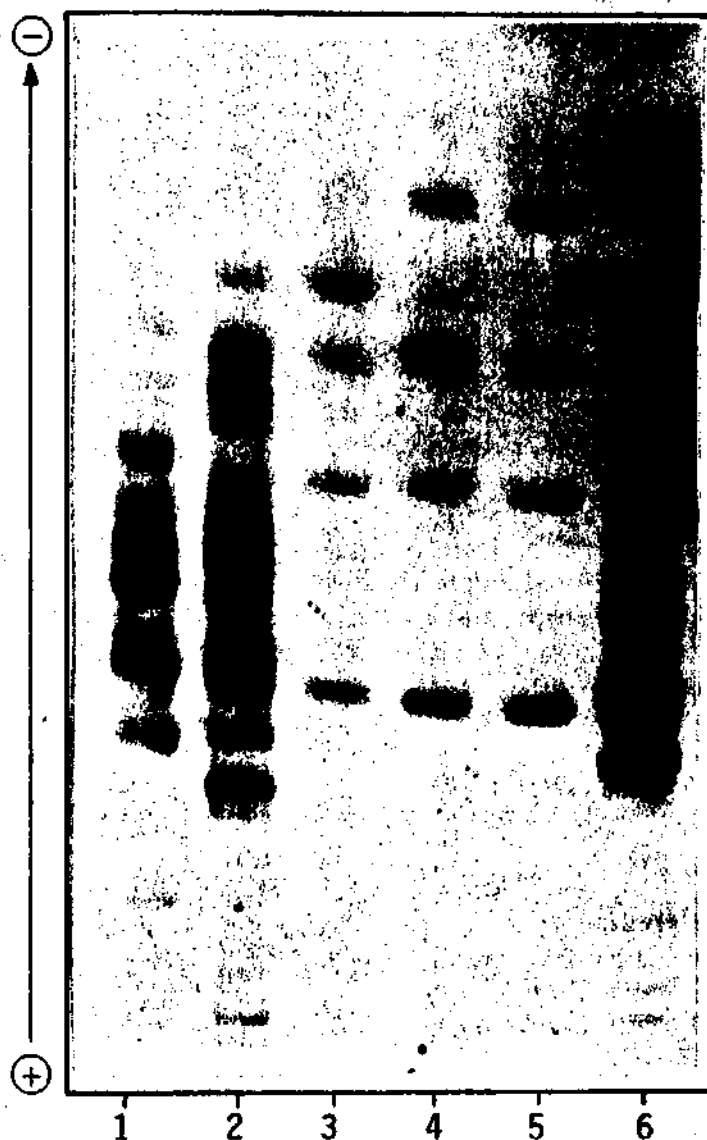


Figure 8. Starch gel electrophoresis (aluminum lactate buffer 0.1 M, pH 3.2, 3 M urea; 15 hour run at 12 V/cm) of the following samples: chloroform-methanol 2:1 (v/v) extract from 1, *T. turgidum* cv. Senatore Capelli; 2, *T. aestivum* cv. Candéal; 6, *T. turgidum* cv. Ledesma; 3, 4, and 5, fractions containing material with MW less than 25,000 from chloroform-methanol 2:1 extracts from the cvs. Candéal, Senatore Capelli and Ledesma, respectively. Gels were stained with 0.6% nigrosine in MeOH-H₂O-HOAc, 5:5:1, for 16 hours. (Aragoncillo and co-workers, unpublished data.)

Three such inhibitors have been extracted from both tetraploid and hexaploid wheat, but no genetic studies have been done.

As noted by Petrucci et al (1974) and Deponte et al (1976), three basic families of α -amylase inhibitors exist in mature wheat kernels. The first two families, whose genetics have been preliminarily investigated, are generally referred to as the 0.19 and 0.28 groups, from their R_f values on PAGE. These inhibitor groups contain proteins of molecular weights (mol wt) of approximately 24,000 and 12,000, respectively. A third family, the inheritance of which has not been studied, contains inhibitors with mol wt around 60,000 (Petrucci et al, 1974). Although these three α -amylase inhibitor families differ widely in the mol wt of their native forms, the 24,000 and 60,000 mol wt forms appear to dissociate into subunits of 12,000 mol wt upon denaturation with SDS or upon reduction of their disulfide bonds with 2-mercaptoethanol (Buonocore et al, 1977; Deponte et al, 1976; Petrucci et al, 1974). This led Buonocore et al (1977) to speculate that all of the albumin α -amylase inhibitors of wheat may be coded by a few closely related genes that may have arisen by mutation from one common ancestor. Some of the inhibitor peptides presumably associated, resulting in the 24,000 and 60,000 mol wt inhibitors, whereas others remained single and comprise the 0.28 family (Buonocore et al, 1977). Petrucci et al (1978) have provided some biochemical evidence for this proposal by determining the amino acid sequence of the first 23 residues of the 0.19 and 0.28 inhibitors. When the inhibitor 0.19 was sequenced without separating its two component peptides, only one residue was detected after each sequencing cycle. Unless one of the two peptides were blocked and not sequenced, this would indicate that the amino terminal sections of the two peptides composing this inhibitor were identical or very similar. Unfortunately, the paper did not report enough quantitative data to ensure that both chains of the protein were indeed being sequenced. The sequences of the 0.19 (Petrucci et al, 1978) and 0.28 (Redman, 1976) inhibitors showed homology after the reading frame was shifted by one amino acid residue at position 4 of the 0.19 protein. It therefore appears likely that the 0.28 protein and the two subunits of the 0.19 inhibitor are indeed specified by genes that evolved from a common ancestor after gene duplication.

Aqueous extracts of several diploid, tetraploid, and hexaploid *Triticum* and *Aegilops* species, which some now consider as a single genus (*Triticum*), have been examined for α -amylase inhibitor activity (Bedetti et al, 1974, 1975; Vittozzi and Silano, 1976). Of the diploid *Triticum* species examined, only *T. urartu* contained any proteins that inhibited either *Tenebrio molitor* (yellow mealworm) or human salivary amylases. *T. urartu* contains a 22,000 mol wt protein that inhibits α -amylase from both sources. It appears, then, that the A genome of wheat does not normally contain genes coding for any active inhibitors that would have been detected by the analytical methods utilized. Johnson (1975) proposed that *T. urartu* was the donor of the B genome to tetraploid and hexaploid wheats, and, with respect to the α -amylase inhibitor contents, it does appear that *T. urartu* is more similar to *Aegilops* species than to the diploid *Triticum* species.

The tetraploid *T. turgidum* (genomes AB) contained amylase inhibitors of molecular weights 60,000, 22,000, and 11,000. Because the A genome donor species does not seem to have had genes coding for active inhibitors, such genes probably came from the B genome parent. Various *Aegilops* species have

repeatedly been proposed as donors of the B genome to the tetraploid and hexaploid wheats, and the seven *Aegilops* species tested all contained one or more α -amylase inhibitor proteins. Of the *Aegilops* species examined, *Ae. longissima* had the inhibitor complement most similar to that of *T. turgidum*, indicating it may have been involved in the evolution of the polyploid wheats. *A. squarrosa*, commonly acknowledged as the donor of the D genome to bread wheat, contained two inhibitor species—one each of 11,000 and 22,000 mol wt. These data are consistent with the probability that the A genome of bread wheat does not contain any genes for active α -amylase inhibitors, whereas both the B and D genomes probably contain at least one gene for the inhibitors.

Konarev (1978) examined several *Triticum* and *Aegilops* species, using antibodies produced against the 0.19 (24,000 mol wt) amylase inhibitor of bread wheat. His results agreed with those discussed above, in that all species of wheat and *Aegilops* reacted with the antiserum except the diploid wheat species *T. boeoticum* and *T. monococcum*. Not surprisingly, *T. urartu* contained a protein that the inhibitor antibodies recognized. The only species that contained proteins that reacted with the 0.19 antibodies, while reportedly not having a 22,000 mol wt inhibitor (Vittozzi and Silano, 1976), was *Ae. speltoides*, which produced a weak precipitin line. Vittozzi reported that *Ae. speltoides* contained only inhibitors of 11,000 and 44,000 mol wt and not of 22,000 mol wt (0.19) protein. This probably means that antibodies raised to the 22,000 mol wt protein can recognize either the 11,000 or 44,000 mol wt entities. If the genes coding for the 11,000 mol wt inhibitor and for the subunits of the 22,000 mol wt inhibitors evolved from a common ancestral gene (Petrucci et al, 1978), the subunits might be similar enough in structure to cross-react with common antibodies.

Only preliminary and inconclusive reports have been published concerning the chromosomal location of genes encoding α -amylase inhibitors, although such data are available for protein components of albumin fractions that may include at least some of the inhibitors. The proteins that may be α -amylase inhibitors are discussed in the following paragraphs.

Pace et al (1978) compared the gel filtration patterns of amylase inhibitors extracted from kernels of 11 compensating nullitetrasonic stocks of Chinese Spring wheat. The analytical procedure they used separated three inhibitor fractions eluting at volumes corresponding to molecular weights of 11,000, 22,000, and 60,000, respectively, but did not permit identification of homoeologous inhibitor variants within each molecular weight class. Under these conditions, structural genes for protein systems that are encoded by duplicate or triplicate homoeologous gene sets are impossible to locate. None of the 11 nullitetrasonic lines observed by Pace et al (1978) had completely lost the ability to synthesize the 22,000 or the 60,000 mol wt inhibitors, indicating either that the genetic control of these inhibitors resides in chromosomes not included in the study or that the proteins are controlled by more than one gene. Only an extremely low level of the 11,000 mol wt inhibitor was present in the nulli 6D tetra 6B line, but no other 6D aneuploids were analyzed. Completely clarifying the findings of Pace et al will require the resolution of possible homoeologous protein variants within each molecular weight class and a complete exploration of each of the three wheat genomes.

Using appropriate antisera, Bozzini et al (1971) concluded that synthesis of two albumins, designated PCS and Mb 0.19 (possibly an amylase inhibitor), was

controlled by chromosomes 3D and 4D, respectively. Noda and Tsunewaki (1972) analyzed buffer-soluble proteins isolated from 20 ditelosomics of Chinese Spring wheat by IEF and found that three major protein components were associated with chromosome arms 3AS, 3BS, and 3DS. They further observed that in IEF gels run with proteins from ditelosomics having one arm absent from chromosomes 4A, 6A, 1B, and 4B, some bands stained less intensely than in gels loaded with proteins from the euploid. Cubadda (1975) used SDS-PAGE to investigate the chromosomal locations of genes controlling the synthesis of seven globulins that precipitated at $(\text{NH}_4)_2\text{SO}_4$ concentrations between 0.8M and 1.2M. Of the seven proteins, the synthesis of one was controlled by chromosome 3A, two were controlled by 3B, and the other four were controlled by chromosome 3D.

Rodriguez-Loperena et al (1975a) showed that several protein components of mol wt less than 25,000, normally extracted from wheat with 70% ethanol, could also be extracted directly from wheat with water. Aragoncillo et al (1975b) then investigated the chromosomal locations of the genes encoding these proteins. Genes controlling proteins 6, 7, 14, and 15 were assigned to the short arm of chromosome 3B, the protein 5 gene(s) to the short arm of chromosome 3D, the gene for protein 1 to chromosome 5D, and protein 10 was controlled by a gene on the short arm of chromosome 6B. Waines (1973) had previously investigated the same protein fractions by PAGE (pH 4.3) but was unable to resolve all the individual protein components. He found that the absence of chromosome arm 3D altered the intensity of a complex band (presumably containing at least two components) also present in *Ae. squarrosa* and that the presence of a second band was correlated with chromosome 3B.

Some reviewers (Buonocore et al, 1977; Kasarda et al, 1976a) suggested that one or more of the proteins genetically controlled by chromosome 3D (Cubadda, 1975) are identical to the 0.19 α -amylase inhibitor. The same was also proposed for protein 5 of Aragoncillo et al (1975b). If the proposal is true, it implies that the assignment of the gene coding for the 0.19 albumin to chromosome arm 4D by Bozzini et al (1971) was erroneous. This interpretation seems plausible, but it should be studied further. Taking into account their isoelectric points, electrophoretic mobilities, and solubilities, protein 5 of Aragoncillo et al (1975b) is probably identical to the protein whose control was assigned by Noda and Tsunewaki (1972) to the same chromosome arm. However, this protein seems to differ from the PCS albumin (Bozzini et al, 1971), which has an isoelectric point much higher than that of protein 5. Proteins 6 and 7 on the two-dimensional map of Aragoncillo et al (1975b) probably are the same as the proteins in band 4 of the IEF pattern of Noda and Tsunewaki (1972), whereas the protein components 14 and 15 are probably equivalent to those comprising band 3. Finally, the component 1 protein of Aragoncillo et al (1975b) is probably the same as an albumin with a gene assigned by Shepherd to chromosome 5D (Konzak, 1977).

Protein 5, whose control was assigned to chromosomal arm 3DS by Aragoncillo et al (1975b), was replaced by two proteins (a_3b_3) in 3D-3Ag wheat-*Agropyron* and 3D-3R wheat-rye substitution lines (Rodriguez-Loperena et al, 1975b). Protein(s) a_3b_3 from *Agropyron elongatum* or from *Secale cereale* had the same peculiar solubility properties as protein 5 from wheat: they were readily extractable by 70% ethanol and by water but not by chloroform-methanol mixtures. Genes for proteins a_3b_3 and 5 were further mapped by analysis of the

proteins present in plants containing 3D-3Ag recombinant chromosomes in transfer lines synthesized by Sears (1973). Rodriguez-Loperena et al (1975b) concluded that genes encoding these proteins were located in homoeologous positions on the S-arms of chromosomes 3D (protein 5) and 3Ag (protein a₃b₃) near the centromeres, because transfer lines carrying large terminal segments of the 3AgS arm showed the wheat phenotype, whereas in a transfer line with an internal 3Ag segment, protein 5 was replaced by a₃b₃.

IX. REGULATORY AND QUANTITATIVE GENETIC EFFECTS

Progress in the characterization of regulatory genes that control the expression of structural genes for specific endosperm proteins has been rather limited. Some of the original findings in this field were later shown to be spurious, and confirmation of others is pending. Considerable research has been conducted to determine the chromosomal locations of genes affecting the total endosperm protein content of wheat. Most have made use of aneuploids and related stocks. Many of the reports relating to this topic have been misleading, however, because no account was taken of the fact that protein percentage is often negatively correlated with yield. For example, the results of a cooperative study compiled by Law and Brown (1978) clearly demonstrated that many ditelocentric lines that showed greatly increased protein percentages over euploid Chinese Spring wheat did not differ significantly from it after appropriate corrections were introduced to compensate for differences in yield.

A. Redundancy and Expression Levels of Genes Encoding Endosperm Proteins

A linear correlation between structural gene dosage and the amount (or activity) of the corresponding protein has been observed in most eucaryotic systems investigated, although systems that do not show this relationship have also been reported (Aragoncillo et al, 1978). A peculiar gene dosage situation exists in hexaploid wheat. Several lines of evidence indicate that a diploidization process starts after the polyploid forms. The main features of the process are a change to a diploid meiotic behavior, achieved either by the structural rearrangement of the chromosomes or by the action of diploidizing genes; the evolution of some redundant genes toward different functions or different developmental specificities; and the loss of redundant genetic activity. The last two aspects of the process result in a reduction of the effective gene dosage of the systems involved (Aragoncillo et al, 1978). Based on a survey of data on chromosomal location of genes encoding 28 biochemical systems, García-Olmedo et al (1978b) estimated the proportions of systems controlled by triplicate, duplicate, and single loci (Table I). Most of the triplicate loci corresponded to genes controlling enzyme systems, whereas most of the incomplete homoeologous sets (duplicate or single loci) were associated with genes coding for endosperm proteins that have no apparent enzymatic functions. Dosage effects have been repeatedly observed during investigations of chromosome-protein associations in wheat. A more quantitative study of gene-dosage responses was conducted by Aragoncillo et al (1978) with a group of six endosperm proteins encoded by incomplete (not triplicate) homoeologous gene

sets. Approximately linear dosage responses were observed for all the proteins. For two of the proteins, however, 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 (Fig. 9) imply 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 (1978b) showed differences in gene-dosage responses among alleles at a locus encoding protein CM3 and CM3' in *T. turgidum*. The net number of protein molecules present 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 CM3 as CM3' 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.

One important consequence of gene redundancy is the possibility of positive and negative heterotic interactions between homoeoalleles. An allopolyploid is in fact a "permanent heterozygote" in which a variety of intergenomic interactions can occur at the molecular level (García-Olmedo et al, 1976). The possible implications of changes in gene-dosage responses during the evolution of the cultivated cereals as related to the overall protein composition of cereal endosperm were discussed recently by García-Olmedo and Carbonero (1981).

B. Regulation of Gliadins and Glutenins by Group 2 Chromosomes

Some proteins are reportedly undetectable in the endosperms of stocks tetrasomic for chromosomes that are different from those carrying structural genes for those proteins. This was confirmed, however, only in certain proteins affected by the group 2 chromosomes. For example, Orth and Bushuk (1974) reported the repression of the synthesis of some glutenin subunits by four doses of chromosome 2B, 3B, or 6B. Subsequent work by Bietz et al (1975), however, did not confirm these findings. Similarly, Aragoncillo et al (1975b) concluded

TABLE I
Redundancy of Genes Encoding Biochemical Markers in Wheat

Redundancy	Sets		Genomes		
	Number	percent	A	B	D
Triplicate ^a	16	57	+	+	+
Duplicate ^b	7	25	+	+	-
			+	-	+
			-	+	+
Single ^b	5	18	+	-	-
			-	+	-
			-	-	+
Percent silenced loci			32	18	11

^a Mostly enzymes.

^b Mostly endosperm proteins of presumed reserve function.

that the structural gene for the nongliadin component 2 of a 70% ethanol extract was apparently repressed by four doses of chromosome 7B in the absence of chromosome 7D. However, this observation was possibly made because segments of chromosome 6B from the cultivar Hope were present in the nulli 7D-tetra 7B Chinese Spring stock analyzed. In a stock provided by Dr. E. R. Sears that had undergone two further backcrosses to Chinese Spring, the protein was present in amounts equal to that found in the euploid wheat.

Shepherd (1968) observed that the one-dimensional SGE pattern of gliadins from tetra 2A and nulli 2D-tetra 2A Chinese Spring stocks lack protein(s) with structural gene(s) seemingly located in chromosome 6D. He postulated that chromosome 2A carries an inhibitor gene that, when present in four doses,

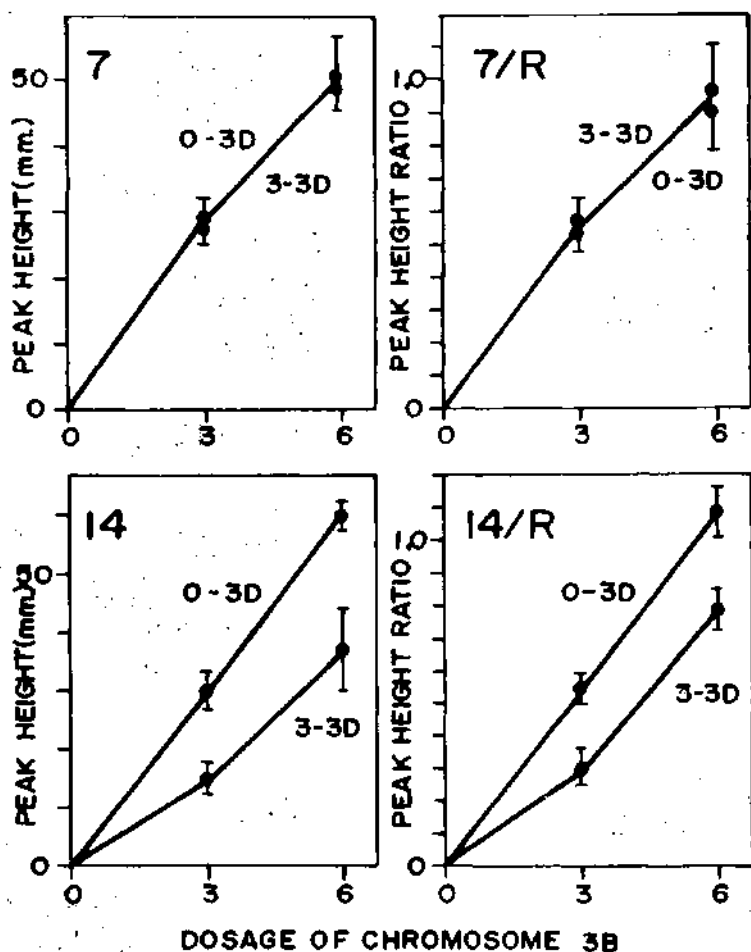


Figure 9. Chromosome-dosage responses for proteins 7 and 14, encoded by genes located in chromosome 3B: Nulli 3B-tetra 3A and nulli 3B-tetra 3D (0 doses of structural genes), euploid (3-3A, 3-3B, 3-3D), nulli 3D-tetra 3A (6-3A, 3-3B, 0-3D), nulli 3A-tetra 3B (0-3A, 6-3B, 3-3D), nulli 3D-tetra 3B (3-3A, 6-3B, 0-3D). Values for 7 or 14 in nulli 3A-tetra 3D (0-3A, 3-3B, 6-3D) and in the euploid (3-3A, 3-3B, 3-3D) did not differ significantly (measured in a separate experiment; not represented in the graphs). R = sum of peak heights of proteins 16 and 17. Dosage responses for both proteins and differences at each dosage for protein were significant ($P < 0.05$). Differences at each dosage for protein 7 were not significant. (Protein 5 could not be measured for technical reasons.) (Aragoncillo et al. 1978. Reproduced with permission of the U.S. Acad. Sci.).

specifically inhibits the production of those gliadins encoded by chromosome 6D. Waines (1973) found evidence that group 2 chromosomes are involved in the control of some gliadins. However, his protein separation method was optimized for the lower molecular weight components of the 70% ethanol extract, and the classical gliadins were poorly resolved. More recently, Brown and Flavell (1981) reinvestigated this problem using a two-dimensional protein separation technique that gives a higher resolution than that of Waines (Brown et al 1979). They confirmed the observations of Shepherd (1968) that tetra 2A and nulli 2D-tetra 2A wheat stocks lack two gliadin components encoded by genes located in the α arm of chromosome 6D. However, different stocks of the nulli 2B-tetra 2A line, which was not analyzed by Shepherd (1968), yielded different phenotypes. Therefore, no simple regulation model can be proposed based on these observations. The authors did not exclude the possibility that the observed effects may be the results of deletions occurring during the production and/or maintenance of the critical stocks. The contention that group 2 chromosomes may have regulatory properties is further supported by findings with different substitution lines (Brown and Flavell, 1981). Of 32 group 2 intervarietal substitution lines examined, only two—Chinese Spring/ Hope 2D and Chinese Spring/ Timstein 2D—showed close similarities to the tetrasomic 2A line. The simplest interpretation for this finding is that the varieties Hope and Timstein contain alleles on chromosome 2D, the regulatory effects of which are similar to those of chromosome 2A of Chinese Spring wheat.

C. Location of Genes Affecting Total Protein

The problems and techniques involved in locating genetic factors in wheat that affect quantitative characters were thoroughly studied by Law (1966). Both protein content and protein composition are difficult to manipulate in genetic studies and in breeding programs (Johnson et al, 1968, 1973; Law and Brown, 1978). For example, the percentage of protein in wheat is generally negatively correlated with yield and percentage of lysine. This means that appropriate corrections must be made before a meaningful analysis of the data can be conducted. After a genetic effect is well established, it is often difficult to determine whether it is truly a regulatory effect or an indirect pleiotropic effect. For example, altering kernel size often indirectly affects the percentage of protein in the kernel. Such problems have undoubtedly been responsible for much of the confusion relating to this topic. Because of such problems we will examine the effects associated with the chromosomes of groups 2 and 5, two of the cases that have been studied most thoroughly.

Jagannath and Bathia (1972) found that when the pair of 2R chromosomes of rye is substituted for any of their homoeologous chromosome pairs in Chinese Spring wheat, cereal lines result that contain elevated percentages of protein in their grains. Law et al (1978a, 1978b) substituted the 2M chromosomes from *Aegilops comosa*, the 2C^u chromosomes from *Ae. umbellulata*, and the 2R^m chromosomes from *Secale montanum* for their related (2A, 2B, and 2D) chromosomes in Chinese Spring wheat and investigated the effects these alterations had on the grain proteins. Using these substitution lines, the group 2 chromosomes could be ranked according to their ability to promote production of grain protein: 2A > 2M \geq 2B > 2C^u > 2D > 2R^m. The substitution of

chromosome 2M for 2D resulted in an increased protein content, whereas the 2M for 2A substitution line contained less protein than euploid Chinese Spring. Although the differences caused by homoeologous chromosomes may not all hold in other wheat varieties, the substitution of 2M for 2D chromosomes in two commercial wheat cultivars also resulted in increases in the protein content of the varieties (Law et al, 1978a, 1978b).

Atlas 66 was identified as a high-protein wheat variety by Middleton et al (1954) and has subsequently been used in different breeding programs. Morris et al (1973, 1978) investigated the chromosomal locations of the gene(s) responsible for the increased protein content of this variety. They investigated F₂ plants from crosses of Wichita monosomics and Atlas 66-Wichita high protein lines and concluded that the Atlas 66 group-5 chromosomes contributed genes for high protein (Morris et al, 1973). By studying substitutions of the 5A, 5B, and 5D chromosomes of Atlas 66 into Chinese Spring, they showed that the 5D (Morris et al, 1973) and 5A (Morris et al, 1978) chromosomes carried genes for increased protein, although the 5D gene caused the major effect. Law et al (1978a) conducted a detailed study of the genetic variation associated with chromosome 5D and showed that at least two genes, *Pro1* and *Pro2*, were involved. *Pro1* appeared identical to gene *Vrn3*, the gene for a vernalization requirement, and was located on the long arm of chromosome 5D. The gene *Pro2* was not closely linked with either *Vrn3* or *Pro1* and was assigned to the short arm, close to the gene *Ha*, which controls grain hardness.

X. A CATALOG OF CHROMOSOME-PROTEIN RELATIONSHIPS

The chromosomal locations assigned to genes encoding wheat endosperm proteins and to genes that appear to alter the expression of these structural genes are catalogued in Table II. There seems to be general agreement regarding which chromosomes are involved in the genetic control of the different protein groups. However, some discrepancies exist that in some cases probably arise from differences in the biochemical methodology (extraction, fractionation, staining, etc.) and in other cases probably arise from improper protein identification or contamination or mislabelling of genetic stocks.

XI. CONCLUSIONS AND PERSPECTIVES

Significant advances have been made in the study of the genetic control of wheat endosperm proteins, and at least tentative assignments of the chromosomal locations of many genes affecting major proteins have been achieved. Genetic analysis has been helpful in demonstrating the complexity of the endosperm protein composition, because similar proteins that were very difficult or impossible to separate from each other by biochemical methods have been separated by the use of genetically altered wheat lines. We hope that the analysis of more thoroughly characterized major endosperm proteins in genetic stocks of well-verified chromosome composition will soon lead to a more complete knowledge of this important source of dietary protein. One important practical aspect that will require further research is the extension of chromosome-protein relationships to include a third variable—quality traits. Studies relating to the functionality of wheat flour components (Pomeranz,

TABLE II

Locations of Genes that Control Endosperm Proteins in Wheat and Related Species

Protein(s) Controlled ^{a,c}	Chromosomal Location ^b	Genetic Material ^c	Methods ^c (Extraction/ Separation)	References
Gliadins				
Two bands (ω)	IDS	DT;CS	Lactate buffer, pH 3.2, 2M urea; SGE (pH 3.2)	Boyd and Lee, 1967; Boyd et al, 1969
K ₁ , L ₁ ^d , L ₂ ^d , L ₇ ^d	IAS	NT, DT, T; CS	2M urea/SGE (pH 3.2)	Shepherd, 1968
K ₃ , K ₄ , M ₂ , L ₂ ^d , L ₃ ^d	IBS			
J ₂ , J ₃ , L ₁ ^d , L ₃ ^d	IDS			
M ₆ , M ₇	6AS			
L ₄ ^d	6BS			
M ₅	6DS			
Regulatory ^c	2A			
Some bands	V (1R)	rye/wheat addition lines: King/ Holdfast, Imperial/CS		
3, 4, 5 ^d , 6, 7, TH	1A (7D)	CS/TC substitution lines	70% ethanol/SGE (pH 3.2)	Solari and Favret, 1970
5 ^d , 7 ^d , 8, 12, 13, TH	1B (2B)			
25, 26, CS	2A			
24 CS	2D			
25, 26, CS	6A			
22, CS	6D			
Number of components				
Three	1A	NT;CS	2M urea, 20% sucrose/IEF (pH 5-9) × SGE (pH 3.2)	Wrigley and Shepherd, 1973
Six	1B			
Four	1D			
Five	6A			
Ten	6B			
Five	6D			
Several bands	A (6C ^u) B (1C ^u)	<i>Aegilops umbellulata</i> /CS addition lines	2M urea/SGE (pH 3.2)	Shepherd, 1973

TABLE II (continued)

Protein(s) Controlled ^{a,c}	Location ^b	Genetic Material ^c	Extraction: Separation ^c	References
Five bands	E (1R)	Progeny of double monosomics: 20'' + 1'1D + 1'1R		Shepherd, 1973
Bands 39 mm and 37 mm Band 35 mm	2A 6A 2D 6D	NT; CS	70% ethanol/ PAGE (pH 4.3)	Waines, 1973
Several bands	1A, 1B, 1D, 6A	Odesskaya variety		Rybalko, 1975
One band CNN, one band CS	1A	CS/CNN substitution lines	8.5 mM lactate buffer/ PAGE (pH 3.2)	Kasarda et al, 1976b
Six bands CNN, four bands CS	1B			
Three bands CNN, three bands CS	1D			
A-gliadins: 3CH, 2CS	6A			
One band CS	6B			
Several bands	Groups 1 & 6	DT, NT; CS	2M urea/ PAGE	Mitrofanova, 1976
Several bands	1B, 1D, 4A ^c , 6B	M CS × Kavkaz	70% ethanol/ SGE (pH 3.1)	Sasek and Kösner, 1977
Several bands	Groups 1 & 6	NT, DT, CS; M CS × Odesskaya	SGE	Sozinov et al, 1978
Number of components				
Five	1BS	NT, T, DT, CS interspecific substitution	2M urea, 0.5% SDS, 0.6%	Brown et al, 1979; Brown
Five	1DS	lines chromosome groups,	2-mercaptoethanol/IEF	and Flavel, 1981;
Two	6AS	1,2,6 <i>Ae. comosa</i> , <i>Ae.</i>	(pH 4.0-7.5) × SDS-PAGE	Brown et al, 1981
One	6BS	<i>umbellulata</i> , <i>S. montanum</i>		
Two	6DS	and intervarietal substitution		
		lines from eight varieties		
Regulatory ^c	2A	T2A, N2DT2A; CS/ Hope 2D, CS/ TST 2D		
Regulatory ^c	1A, 1B, 1D	Intervarietal substitution lines		
Six components	1C ^a	CS- <i>Ae. umbellulata</i> group 1 substitution lines		

R4	IRS	CS-Imperial rye addition lines	0, 1 SDS, 1% 2-mercaptoethanol/ SDS-PAGE (pH 8.8)	Lawrence and Shepherd, 1981
U4	IC ^o S	CS- <i>Ae. umbellulata</i> addition lines		
B2, B3	5S (barley)	CS-barley cultivar Betzes addition lines		
Several bands	I ¹ (1AgS)	CS- <i>Agropyron elongatum</i> addition lines	2M urea/SGE (pH 3.1)	
Glutenins				
Components with mol wt 152,000; 112,000; 60,000; 45,000 ^d	IDL	NT, DT, CS; tetraploid LD222 and its 1B/1D substitution line	(0.1M acetic acid, 3M urea, 0.01M HTAB)/SDS-PAGE (pH 7.3)	Orth and Bushuk, 1974
Regulatory ^e	2B, 3B, 6B			
Components with mol wt 104,000; 93,000; 133,000; 86,000; 68,000 (albumin) ^e	IBL IDL 4DL	NT, DT, CS/CNN substitute lines; tetraploids and diploids	Sequential extraction, final step: 1% 2-mercaptoethanol, 0.1% SDS (pH 8.9)/SDS-PAGE (pH 8.9)	Bietz et al, 1975
Regulatory	Not found			
Components with mol wt 125,000; 88,000	IDL	NT, T, DT, CS; interspecific substitution lines; etc.	2M urea, 0.5% SDS, 0.6% 2-mercaptoethanol/IEF (pH 4.0-7.5) × SDS-PAGE	Brown et al, 1979; Brown and Flavell, 1981; Brown et al, 1981
Number of components				
0-1	1AL	NT, DT; variation in 98	0.1 SDS, 1% 2-mercaptoethanol/ SDS-PAGE (pH 8.8)	Lawrence and Shepherd, 1980, 1981
1-2	IBL	hexaploid cultivars, some		
2	IDL	intervarietal substitution		
R1 and R2	1RL	CS-Imperial rye addition lines		
R3 and R?	2R			
U1, U2	B (IC ^o)	CS- <i>Ae. umbellulata</i> addition lines		
E1, E2	I (1AgL)	CS- <i>Ag. elongatum</i> addition lines		
B1	5L (barley)	CS-Barley cultivar Betzes addition lines		

TABLE II (continued)

Protein(s) Controlled ^{a,c}	Location ^b	Genetic Material ^e	Extraction/ Separation ^f	References
Purothionins				
β	1AL	NT, DT; CS	Petroleum ether + HCL-ethanol/ SGE (pH 3.2) or 0.05N H ₂ SO ₄ / SGE (pH 5.2) \times SGE (pH 3.2)	Garcia-Olmedo et al, 1976; Fernandez de Caleyá et al, 1976;
$\alpha_B (= \alpha_1)$	1BL			
$\alpha_D (= \alpha_2)$	1DL			
Lipid component (DGDG)	5DS			Hernandez-Lucas et al, 1977a; Carbonero et al, 1978
R	1RL	CS-Imperial rye addition lines; Aurora and Kavkaz varieties		Sanchez-Monge et al, 1979
CM Proteins				
16; CM3 (12-13) ^d	4A β	NT, DT, CS; Blau-Korn (4A/5R substitution)	70% Ethanol or chloroform- methanol (2:1)/SGE (pH 3.2) or IEF (pH 5-8) \times SGE (pH 3.2)	García-Olmedo and García- Faure, 1969; García- Olmedo and Carbonero, 1970; Aragoncillo et al, 1975b; Rodríguez-Loperena et al, 1975b
17	4D			
CM2 (8-9)	7BS			
11, CM1 (3-4) a ₇ ; b ₇ ; c ₇	7DS ^f 7AgS ^f	Argus (7D/7Ag substitution); 7D/7Ag transfer lines		
Bands				
69 mm ^d ; 83 mm ^a	4A	NT, CS	70% Ethanol/PAGE (pH 4.3)	Waines, 1973
65 mm ^d	4D			
90-100 mm	7B			
105 mm	7D			
Low molecular weight gliadins				
1, 6	4B	NT, DT, CS; Blau-Korn (4A/5R substitution)	70% Ethanol or chloroform- methanol (2:1)/10% PAGE (pH 9.0) \times SGE (pH 3.2)	Salcedo et al, 1980a
2	7AS			
3, 4	7DL			
Albumins				
PCS	3D	M; CS	Immunoprecipitation	Bozzini et al, 1971
Mb 0.19	4D ^{e,g}			
Bands				
5 ^{d,e}	1B	DT; CS	Tris-HCl (pH 8.0)/IEF (pH 5-8)	Noda and Tsunewaki, 1972
3	3AS			
3, 4	3BS			
7	3DS			
4 ^d , 5 ^{d,e}	4A			
4 ^d , 5 ^{d,e}	6A			

Bands				
4	3A	NT; CS	0.8-1.2M (NH ₄) ₂ SO ₄ (pH 6.1); PAGE (SDS ^c ; pH ^e)	Cubadda, 1975
6, 7	3B			
1, 2, 3, 5	3D			
Components				
6, 7, 14, 15	3BS	NT, DT; CS	70% Ethanol or H ₂ O/ IEF (pH 5-8) × SGE (pH 3.2)	Aragoncillo et al, 1975b Rodriguez-Loperena et et al, 1975b
5	3DS ^f	CS-Ag. <i>elongatum</i> 3Ag/3D		
a ₃ b ₃	3Ag ^g S	substitution and transfer lines		
a ₃ b ₃	3R	KKF/rye cultivar Dakold		
1	5D	3D/3R substitution line		
2, 10	6BS			
1 Complex band ^d	3D	NT; CS	70% Ethanol/ PAGE (pH 4.3)	Waines, 1973
1 Band	5D	NT; CS		Konzak, 1977
Unclassified				
Several bands ^d	1A, 1B, 1D 2B, 2D 3A, 3D 5A, 5B, 5D 6A, 6B, 6D	DT; CS		Khrabrova et al, 1973
Components with mol wt				
50,000	1AS			Brown and Flavell, 1981
53,000	1DS			

^a Protein designations are usually those proposed by each author.

^b Chromosome or chromosomal arm.

^c N = nullisomic, M = monosomic, T = tetrasomic, NT = compensating nulli-tetrasomic, DT = ditelosomic; Wheats: CS = Chinese spring, CNN = Cheyenne, TC = Thatcher, TST = Timstein, KKF = Kharkof; Method: SGE = starch-gel electrophoresis; PAGE = polyacrylamide gel electrophoresis; IEF = isoelectric focusing; SDS = sodium dodecyl sulfate; HTAB = hexadecyl trimethyl ammonium bromide.

^d No complete disappearance observed upon deletion of chromosome or chromosomal arm.

^e Tentative.

^f Proximal to centromere.

^g See discussion of coding for chromosome arm 4D on p. 29.

1971) may eventually extend to the level of individual protein components and their genetic variants. A different perspective of both theoretical and practical interest is the possible use of endosperm proteins, isozymes, and other easily identified monogenic phenotypic traits as genetic markers. The chromosome structures of species related to wheat have been investigated in this vein (Delibes et al, 1977b, 1981; Hart et al, 1976; Lawrence and Shepherd, 1981; Rodriguez-Loperena et al, 1975b; Sanchez-Monge et al, 1979; Sears, 1977b; Shepherd, 1973). Biochemical markers have been used to directly follow the genetic transfer of agronomic characters, such as disease resistance, from alien species into wheat (Delibes et al, 1977a, 1981; Poperelya and Babayants, 1978; Sears, 1973, 1977b).

ACKNOWLEDGMENTS

F. García-Olmedo and P. Carbonero wish to acknowledge grants from the Fundación Ramón Areces and the Comisión Asesora de Investigación Científica y Técnica (Min. Univ. e Inv., Spain), as well as help from Drs. C. Aragoncillo, G. Salcedo, and C. Hernández-Lucas. The authors thank Drs. R. Morris and E. Sanchez-Monge for reviewing the manuscript and for helpful suggestions.

LITERATURE CITED

- ARAGONCILLO, C., RODRIGUEZ-LOPERENA, M. A., CARBONERO, P., and GARCIA-OLMEDO, F. 1975a. Nigrosine staining of wheat endosperm proteolipid patterns on starch gels. *Anal. Biochem.* 63:603-606.
- ARAGONCILLO, C., RODRIGUEZ-LOPERENA, M. A., CARBONERO, P., and GARCIA-OLMEDO, F. 1975b. Chromosomal control of nongliadin proteins from the 70% ethanol extract of wheat endosperm. *Theor. Appl. Genet.* 45:322-326.
- ARAGONCILLO, C., RODRIGUEZ-LOPERENA, M. A., SALCEDO, G., CARBONERO, P., and GARCIA-OLMEDO, F. 1978. Influence of homeologous chromosomes on gene-dosage effects in allohexaploid wheat (*Triticum aestivum* L.). *Proc. Natl. Acad. Sci.* 75:1446-1450.
- ARAGONCILLO, C., SANCHEZ-MONGE, R., and SALCEDO, G. 1981. Two groups of low molecular weight hydrophobic proteins from barley endosperm. *J. Exp. Bot.* 32:1279-1286.
- AUTRAN, J.-C., LEW, E. J.-L., NIMMO, C. C., and KASARDA, D. D. 1979. N-terminal amino acid sequencing of prolamins from wheat and related species. *Nature* 282:527-529.
- BALLS, A. K., HALE, W. S., and HARRIS, T. H. 1942. A crystalline protein obtained from a lipoprotein of wheat flour. *Cereal Chem.* 19:279-288.
- BEDETTI, C., BOZZINI, A., SILANO, V., and VITTOZZI, L. 1974. Amylase protein inhibitors and the role of *Aegilops* species in polyploid wheat speciation. *Biochim. Biophys. Acta* 360:299-307.
- BEDETTI, C., BOZZINI, A., POCCHIARI, F., SILANO, V., and VITTOZZI, L. 1975. Biochemical studies on the phylogenesis of α -amylase protein inhibitors from wheat seed. Pages 71-77 in: *Genetics and Breeding of Durum Wheat*. G. T. Scarascia-Mugnozza, ed. Library of the Faculty of Agric., Bari, and Natl. Inst. Nutr., Rome.
- BERNARDIN, J. E., KASARDA, D. D., and MECHAM, D. K. 1967. Preparation and characterization of α -gliadin. *J. Biol. Chem.* 242:445-450.
- BIETZ, J. A., and WALL, J. S. 1973. Isolation and characterization of gliadin-like subunits from glutenin. *Cereal Chem.* 50:537-547.
- BIETZ, J. A., and WALL, J. S. 1975. The effect of various extractants on the subunit composition and associations of wheat glutenins. *Cereal Chem.* 52:145-155.
- BIETZ, J. A., SHEPHERD, K. W., and WALL, J. S. 1975. Single-kernel analysis of glutenin: Use in wheat genetics and breeding. *Cereal Chem.* 52:513-532.
- BIETZ, J. A., HUEBNER, F. R., SANDERSON, J. E., and WALL, J. S. 1977. Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. *Cereal Chem.* 54:1070-1083.
- BOWDEN, W. M. 1959. The taxonomy and nomenclature of the wheats, barleys, and ryes and their wild relatives. *Can. J. Bot.* 37:657-684.
- BOYD, W. J. R., and LEE, J. W. 1967. The control of wheat gluten synthesis at the

- genome and chromosome levels. *Experientia* 23:332-333.
- BOYD, W. J. R., LEE, J. W., and WRIGLEY, C. W. 1969. The D-genome and the control of wheat gluten synthesis. *Experientia* 25:317-319.
- BOZZINI, A., CANTAGALLI, P., and PIAZZI, S. F. 1971. Chromosomal location of the genetic control of two proteins specifically attributed to hexaploid wheat by immunochemical methods. *E.W.A.C. Newsl.* 3:16-17.
- BROWN, J. W. S., and FLAVELL, R. B. 1981. Fractionation of wheat gliadin and glutenin subunits by two-dimensional electrophoresis and in gliadin synthesis. *Theor. Appl. Genet.* 59:349-359.
- BROWN, J. W. S., KEMBLE, R. J., LAW, C. N., and FLAVELL, R. B. 1979. Control of endosperm proteins in *Triticum aestivum* (var. Chinese Spring) and *Aegilops umbellulata* by homoeologous group 1 chromosomes. *Genetics* 93:189-200.
- BROWN, J. W. S., LAW, C. N., WORLAND, A. J., and FLAVELL, R. B. 1981. Genetic variation in wheat endosperm proteins. An analysis by two-dimensional electrophoresis using intervarietal chromosomal substitution lines. *Theor. Appl. Genet.* 59:361-371.
- BUONOCORE, V., PETRUCCI, T., and SILANO, V. 1977. Wheat protein inhibitors of α -amylase. *Phytochemistry* 16:811-820.
- CARBONERO, P., and GARCIA-OLMEDO, F. 1969. Purothionins in *Aegilops-Triticum* spp. *Experientia* 25:1110.
- CARBONERO, P., GARCIA-OLMEDO, F., HERNANDEZ-LUCAS, C., and FERNANDEZ de CALEYA, R. 1979. Genetic control of purothionins in wheat: Problems of the aneuploid analysis when searching for regulatory genes. Pages 455-463 in: *Proc. 5th Int. Wheat Genet. Symp.*, New Delhi. S. Ramanujam, ed. Indian Soc. Genet. and Plant Breeding, New Delhi.
- CARBONERO, P., GARCIA-OLMEDO, F., and HERNANDEZ-LUCAS, C. 1980. External association of hordothionin with protein bodies in mature barley. *J. Agric. Food Chem.* 28:399-402.
- CARRASCO, L., VAZQUEZ, D., HERNANDEZ-LUCAS, C., CARBONERO, P., and GARCIA-OLMEDO, F. 1981. Thionins: Plant peptides that modify membrane permeability in cultured mammalian cells. *Eur. J. Biochem.* 116:185-189.
- CHEN, C. H., and BUSHUK, W. 1970. Nature of proteins in Triticale and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. *Can. J. Plant Sci.* 50:9-14.
- COULSON, E. J., HARRIS, T. H., and AXELROD, B. 1942. Effect on small laboratory animals of the injection of the crystalline hydrochloride of a sulfur protein from wheat flour. *Cereal Chem.* 19:301-307.
- CUBADDA, R. 1975. Chromosomal location of genes controlling the synthesis of some soluble proteins in *T. durum* and *T. aestivum*. Pages 653-659 in: *Genetics and Breeding of Durum Wheat*. G. T. Scarascia-Mugnozza, ed. Library of the Faculty of Agric., Bari, and Natl. Inst. Nutr., Rome.
- DANNO, G., KANAZAWA, K., and NATAKE, M. 1974. Extraction of wheat flour proteins with sodium dodecyl sulphate and their molecular weight distribution. *Agric. Biol. Chem.* 38:1947-1953.
- DELIBES, A., DOSBA, F., DOUSSINAULT, G., GARCIA-OLMEDO, F., and SANCHEZ-MONGE, R. 1977a. Resistance to eyespot (*Cercospora herpotrichoides*) and distribution of biochemical markers in hexaploid lines derived from a double cross (*Triticum turgidum* \times *Aegilops ventricosa*) \times *T. aestivum*. Pages 91-98 in: *Interspecific Hybridization Plant Breeding*. E. Sanchez-Monge and F. Garcia-Olmedo, eds. *Proc. 8th Eucarpia Congress*, Madrid.
- DELIBES, A., SANCHEZ-MONGE, R., and GARCIA-OLMEDO, F. 1977b. Biochemical and cytological studies of genetic transfer from the M' genome of *Aegilops ventricosa* into hexaploid wheat. Pages 81-90 in: *Interspecific Hybridization in Plant Breeding*. E. Sanchez-Monge and F. Garcia-Olmedo, eds. *Proc. 8th Eucarpia Congress*, Madrid.
- DELIBES, A., DOSBA, F., OTERO, C., and GARCIA-OLMEDO, F. 1981. Biochemical markers associated with two M' chromosomes from *Aegilops ventricosa* in wheat-*Aegilops* addition lines. *Theor. Appl. Genet.* 60:5-10.
- DEPONTE, R., PARLAMENTI, R., PETRUCCI, T., SILANO, V., and TOMASI, M. 1976. Albumin α -amylase inhibitor families from wheat flour. *Cereal Chem.* 53:805-820.
- DOEKES, G. J. 1968. Comparison of wheat varieties by starch gel electrophoresis of their grain proteins. *J. Sci. Food Agric.* 19:169-176.
- DOEKES, G. J. 1973. Inheritance of gliadin composition in bread wheat. *T. aestivum* L. *Euphytica* 22:28-33.
- DOSBA, F., DOUSSINAULT, G., and RIVOAL, R. 1979. Extraction, identification and utilization of the addition lines *T. aestivum*-*Ae. ventricosa*. Pages 332-337 in: *Proc. 5th Int. Wheat Genet. Symp.*, New Delhi. S. Ramanujan, ed. Indian Soc. Genet. and Plant Breeding.
- EASTIN, J. D., MORRIS, R., SCHMIDT, J. W., MATTERN, P. J., and JOHNSON, V. A.

1967. Chromosomal association with gliadin proteins in the wheat variety Cheyenne. *Crop Sci.* 7:674-676.
- FAVRET, E. A., MANGHERS, L. SOLARI, R., AVILA, A., and MONESIGLIO, J. C. 1970. Gene control of protein production in cereal seeds. Pages 87-95 in: *Improving Plant Proteins by Nuclear Techniques*. IAEA Proc. Series STI/PUB/258, Vienna.
- FELDMAN, M. 1976. Wheats, *Triticum* spp. (Gramineae-Triticineae). Pages 120-128 in: *Evolution of Crop Plants*. N. W. Simmonds, ed. Longman: New York.
- FELDMAN, M. 1979. New evidence on the origin of the B genome of wheat. Pages 120-132 in: *Proc. 5th Int. Wheat Genet. Symp.*, New Delhi. S. Ramanujam, ed. Indian Soc. Genet. and Plant Breeding.
- FERNANDEZ de CALEYA, R., GONZALEZ-PASCUAL, B., GARCIA-OLMEDO, F., and CARBONERO, P. 1972. Susceptibility of phytopathogenic bacteria to wheat purothionins in vitro. *Appl. Microbiol.* 23:998-1000.
- FERNANDEZ de CALEYA, R., HERNANDEZ-LUCAS, C., CARBONERO, P., and GARCIA-OLMEDO, F. 1976. Gene expression in allopolyploids: Genetic control of lipopurothionins in wheat. *Genetics* 83:687-699.
- GARCIA-OLMEDO, F. 1968. Genetics of synthesis of β -sitosterol esters in wheat and related species. *Nature* 220:1144-1145.
- GARCIA-OLMEDO, F., and CARBONERO, P. 1970. Homoeologous protein synthesis controlled by homoeologous chromosomes in wheat. *Phytochemistry* 9:1495-1497.
- GARCIA-OLMEDO, F., and CARBONERO, P. 1981. Chromosomes, genes, proteins and wheat endosperm. In: *Proc. 3rd Int. Wheat Conference*, Madrid. Univ. of Nebraska. In Press.
- GARCIA-OLMEDO, F., and GARCIA-FAURE, R. 1969. A new method for the estimation of common wheat (*T. aestivum* L.) in pasta products. *Lebensm. Wiss. Technol.* 2:94-96.
- GARCIA-OLMEDO, F., SOTELO, I., and GARCIA-FAURE, R. 1968. Identificación de productos de *Triticum aestivum* en las pastas alimenticias. IV. Lipoproteínas solubles en éter de petróleo. *An. Inst. Invest. Agron. Madrid* 17:433-443.
- GARCIA-OLMEDO, F., CARBONERO, P., ARAGONCILLO, C., FERNANDEZ de CALEYA, R., and TORRES, J. V. 1976. Expression of homoeologous molecular systems in wheat allopolyploids. Pages 51-57 in: *Heterosis in Plant Breeding*. A. Janossy and F. G. H. Lupton, eds. *Proc. 7th Eucarpia Congress. Akademiai Kiado, Budapest.*
- GARCIA-OLMEDO, F., CARBONERO, P., ARAGONCILLO, C., and SALCEDO, G. 1978a. Chromosomal control of wheat endosperm proteins. Pages 555-566 in: *Seed protein improvement by nuclear techniques*. IAEA Proc. Series STI/PUB/479. Vienna.
- GARCIA-OLMEDO, F., CARBONERO, P., ARAGONCILLO, A., and SALCEDO, G. 1978b. Loss of redundant gene expression after polyploidization in plants. *Experientia* 34:332-333.
- GARCIA-OLMEDO, F., CARBONERO, P., HERNANDEZ-LUCAS, C., VICENTE, O., and SIERRA, J. M. 1980. Inhibition of the "in vitro" wheat-embryo translation system by a polypeptide from wheat endosperm. (Abstr.) Pages 205-206 in: *Proc. 6th EMBO Symp., Molecular Biologists Look at Green Plants*. EMBL, Heidelberg.
- GARCIA-OLMEDO, F., CARBONERO, P., HERNANDEZ-LUCAS, C., PONZ, F., VICENTE, O., and SIERRA, J. M. 1981. Synthesis and location of thionins in developing endosperms of barley and wheat. In: *Proc. 2nd Seed Protein Symp. Abhdlg. Akad. Wiss. DDR, Abt. Math., Naturwiss. Techn.* In Press.
- HALL, O., JOHNSON, B. L., and OLERED, R. 1966. Evaluation of genome relationships in wheat from their protein homologies. In: *Proc. 2nd Int. Wheat Genet. Symp.*, Lund. J. Mackey, ed. *Hereditas*, Suppl. 2:47-54.
- HART, G. E., McMILLIN, D. E., and SEARS, E. R. 1976. Determination of the chromosomal location of a glutamate oxalacetate transaminase structural gene using *Triticum-Agropyron* translocations. *Genetics* 83:49-61.
- HASE, T., MATSUBARA, H., and YOSHIZUMI, H. 1978. Disulfide bonds of purothionin, a lethal toxin for yeasts. *J. Biochem.* 83:1671-1678.
- HELENIUS, A., and SIMONS, K. 1975. Solubilization of membranes by detergents. *Biochim. Biophys. Acta* 415:29-79.
- HERNANDEZ-LUCAS, C., FERNANDEZ de CALEYA, R., and CARBONERO, P. 1974. Inhibition of brewers' yeasts by wheat purothionins. *Appl. Microbiol.* 28:165-168.
- HERNANDEZ-LUCAS, C., FERNANDEZ de CALEYA, R., CARBONERO, P., and GARCIA-OLMEDO, F. 1977a. Reconstitution of petroleum ether soluble wheat lipopurothionin by binding of digalactosyl diglyceride to the chloroform-soluble form. *J. Agric. Food Chem.* 25:1287-1289.
- HERNANDEZ-LUCAS, C., FERNANDEZ de CALEYA, R., CARBONERO, P., and GARCIA-OLMEDO, F. 1977b. Control of galactosyl diglycerides in wheat endosperm by

- group 5 chromosomes. *Genetics* 85:521-527.
- HERNANDEZ-LUCAS, C., CARBONERO, P., and GARCIA-OLMEDO, F. 1978. Identification and purification of a purothionin homologue from rye (*Secale cereale* L.). *J. Agric. Food Chem.* 26:794-796.
- HUEBNER, F. R., and WALL, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. *Cereal Chem.* 53:258.
- HUNT, L. T., BARKER, W. C., and DAYHOFF, M. O. 1976. Protein data introduction. Pages 21-23 in: *Atlas of Protein Sequence and Structure*, Vol. 5, Suppl. 2. M. O. Dayhoff, ed. National Biomedical Research Foundation, Washington, DC.
- ISLAM, A. K. M. R., SHEPHERD, K. W., and SPARROW, D. H. B. 1975. Addition of individual barley chromosomes to wheat. Pages 260-270 in: *Proc. 3rd Int. Barley Genet. Symp.*, Garching. H. Gaul, ed. Verlag Karl Thieme, Munchen.
- JAGANNATH, D. R., and BATHIA, C. R. 1972. Effect of rye chromosome 2 substitution on kernel protein content of wheat. *Theor. Appl. Genet.* 42:89-92.
- JOHNSON, B. L. 1972. Protein electrophoretic profiles and the origin of the B genome of wheat. *Proc. Natl. Acad. Sci.* 69:1398-1402.
- JOHNSON, B. L. 1975. Identification of the apparent B genome donor of wheat. *Can. J. Genet. Cytol.* 17:21-39.
- JOHNSON, B. L., and HALL, O. 1965. Analysis of phylogenetic affinities in the *Triticinae* by protein electrophoresis. *Am. J. Bot.* 52:506-513.
- JOHNSON, V. A., WHITED, D. A., MATTERN, P. J., and SCHMIDT, J. W. 1968. Nutritional improvement of wheat by breeding. Pages 457-461 in: *Proc. 3rd Int. Wheat Genet. Symp.*, Canberra. K. W. Finlay and K. W. Shepherd, eds. Aust. Acad. Sci.
- JOHNSON, V. A., MATTERN, P. J., SCHMIDT, J. W., and STROIKE, J. E. 1973. Genetic advances in wheat protein quantity and composition. Pages 547-556 in: *Proc. 4th Int. Wheat Genet. Symp.*, Columbia, MO. E. R. Sears and L. M. S. Sears, eds. Agric. Exp. Stn., Univ. of Missouri, Columbia.
- JONES, B. L., and MAK, A. S. 1977. Amino acid sequences of the α -purothionins of hexaploid wheat. *Cereal Chem.* 54:511-523.
- JONES, R. W., TAYLOR, N. W., and SENTI, F. R. 1959. Electrophoresis and fractionation of wheat gluten. *Arch. Biochem. Biophys.* 84:363-376.
- JOPPA, L. R. 1973. Development of disomic substitution lines of durum wheat (*Triticum turgidum* L.). Pages 685-690 in: *Proc. 4th Int. Wheat Genet. Symp.*, Columbia, MO. E. R. Sears and L. M. S. Sears, eds. Agric. Exp. Stn., Univ. of Missouri, Columbia.
- JOPPA, L. R., BIETZ, J. A., and McDONALD, C. 1975. Development and characteristics of a disomic-1D addition line of durum wheat. *Can. J. Genet. Cytol.* 17:355-463.
- JOPPA, L. R., BIETZ, J. A., and WILLIAMS, N. D. 1979. The aneuploids of durum wheat: D-genome addition and substitution lines. Pages 420-426 in: *Proc. 5th Int. Wheat Genet. Symp.*, New Delhi. S. Ramanujam, ed. Indian Soc. Genet. and Plant Breeding.
- KALTSIKES, P. J., EVANS, L. E., and BUSHUK, W. 1968. Durum-type wheat with high bread making quality. *Science* 159:211-212.
- KASARDA, D. D., BERNARDIN, J. E., and NIMMO, C. C. 1976a. Wheat proteins. Pages 158-236 in: *Advances in Cereal Science and Technology*, Vol. 1. Am. Assoc. Cereal Chem., Inc.: St. Paul, MN.
- KASARDA, D. D., BERNARDIN, J. E., and QUALSET, C. O. 1976b. Relationship of gliadin protein components to chromosomes in hexaploid wheats (*Triticum aestivum* L.). *Proc. Natl. Acad. Sci.* 73:3646-3650.
- KERBER, E. R. 1964. Wheat: Reconstitution of the tetraploid component (AABB) of hexaploids. *Science* 143:253-255.
- KHRABROVA, M. A., GALAI, V. S., and MAYSTRENKO, O. I. 1973. Electrophoretic patterns of gluten proteins of some common wheat varieties and of a set of Chinese Spring ditelosomics. Pages 218-232 in: *Cytogenetic Studies on Aneuploids of Common Wheat*. O. I. Maystrenko and V. V. Khvostova; eds. In Russian, English summary. Acad. Sci. U.S.S.R., Siberian Branch, Inst. Cytol. Genet.
- KIMBER, G., and SEARS, E. R. 1968. Nomenclature for the description of aneuploids in the *Triticinae*. Pages 468-473 in: *Proc. 3rd Int. Wheat Genet. Symp.*, Canberra. K. W. Finlay and K. W. Shepherd, eds. Aust. Acad. Sci.
- KIMBER, G., and SEARS, E. R. 1980. Uses of wheat aneuploids. Pages 427-443 in: *Polyploidy, Biological Relevance*. W. H. Lewis, ed. Plenum Publ. Corp.: New York.
- KNEEN, E., and SANDSTEDT, R. M. 1943. An amylase inhibitor from certain cereals. *J. Am. Chem. Soc.* 65:1247.
- KONAREV, A. V. 1978. Identification of albumin 0.19 in grain proteins of cereals. *Cereal Chem.* 55:927-936.
- KONZAK, C. F. 1977. Genetic control of the content, amino acid composition, and processing properties of proteins in wheat. *Adv. Genet.* 19:407-582.

- KRAMER, K. J., KLASSEN, L. W., JONES, B. L., SPFIRS, R. D., and KAMMER, A. E. 1979. Toxicity of purothionin and its homologues to the tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae). *Toxicol. Appl. Pharmacol.* 48:179-183.
- LAW, C. N. 1966. The location of genetic factors affecting a quantitative character in wheat. *Genetics* 53:487-498.
- LAW, C. N., and BROWN, J. W. S. 1978. The cooperative programme to study protein differences among the ditelocentric lines of the wheat variety Chinese Spring. Pages 427-454 in: *Seed Protein Improvement by Nuclear Techniques*. IAEA Proc. Series STI/PUB/479. Vienna.
- LAW, C. N., YOUNG, C. F., BROWN, J. W. S., SNAPE, J. W., and WORLAND, A. J. 1978a. The study of grain protein control in wheat using whole chromosome substitution lines. Pages 483-502 in: *Seed Protein Improvement by Nuclear Techniques*. IAEA Proc. Series STI/PUB/479. Vienna.
- LAW, C. N., WORLAND, A. J., CHAPMAN, V., and MILLER, T. E. 1978b. Homoeologous chromosome transfers into hexaploid wheat and their influence on grain protein amounts. Pages 73-80 in: *Interspecific Hybridization in Plant Breeding*. E. Sanchez-Monge and F. García-Olmedo, eds. Proc. 8th Eucarpia Congress, Madrid.
- LAWRENCE, G. J., and SHEPHERD, K. W. 1980. Variation in glutenin protein subunits of wheat. *Aust. J. Biol. Sci.* 33:221-233.
- LAWRENCE, G. J., and SHEPHERD, K. W. 1981. Chromosomal location of genes controlling seed proteins in species related to wheat. *Theor. Appl. Genet.* 60:333-337.
- LONGWELL, J. R., Jr., and SEARS, E. R. 1963. Nullisomics in tetraploid wheat. *Am. Nat.* 97:401-403.
- MAK, A. S. 1975. Studies of the primary structures of purothionins and purothionin-like proteins from cereals. Ph.D. thesis. Univ. of Manitoba, Winnipeg.
- MAK, A. S., and JONES, B. L. 1976a. Separation and characterization of chymotryptic peptides from α - and β -purothionins of wheat. *J. Sci. Food Agric.* 27:205-213.
- MAK, A. S., and JONES, B. L. 1976b. The amino acid sequence of wheat β -purothionin. *Can. J. Biochem.* 54:835-842.
- MANGHERS, L. E., SOLARI, R. M., AVILA, A., and FAVRET, E. A. 1973. Implications of the genetics of cereal seed proteins in induced mutagenesis. Pages 305-320 in: *Nuclear Techniques for Seed Protein Improvement*. IAEA Proc. Series STI/PUB/320. Vienna.
- MARSHALL, J. J. 1975. α -Amylase inhibitors from plants. *Am. Chem. Soc. Symp. Ser.* 15:244-266.
- MECHAM, D. K., KASARDA, D. D., and QUALSET, C. O. 1978. Genetic aspects of wheat gliadin proteins. *Biochem. Genet.* 16:831-853.
- MEREDITH, P. 1965a. On the solubility of gliadinlike proteins. I. Solubility in non-aqueous media. *Cereal Chem.* 42:54-63.
- MEREDITH, P. 1965b. On the solubility of gliadinlike proteins. II. Solubility in aqueous acid media. *Cereal Chem.* 42:64-148.
- MEREDITH, P. 1965c. On the solubility of gliadinlike proteins. III. Fractionation by solubility. *Cereal Chem.* 42:149-160.
- MEREDITH, P., SAMMONDS, H. G., and FRAZER, A. C. 1960. Examination of wheat gluten by partial solubility methods. I. Partition by organic solvent. *J. Sci. Food Agric.* 11:320-328.
- MIDDLETON, G. K., BODE, C. E., and BAYLES, B. B. 1954. A comparison of the quantity and quality of protein in certain varieties of soft wheat. *Agron. J.* 46:500-502.
- MIFLIN, B. J., and SHEWRY, P. R. 1979. The biology and biochemistry of cereal seed prolamins. Pages 137-158 in: *Seed Protein Improvement in Cereals and Grain Legumes*, Vol. I. IAEA Proc. Series STI/PUB/496. Vienna.
- MIFLIN, B. J., BYERS, M., FIELD, J. M., FAULKS, A. J., and FORDE, J. 1980. The isolation and characterization of proteins extracted from whole milled seed, gluten, and developing protein bodies of wheat. *Ann. Technol. Agric.* 24:133-147.
- MINETTI, M., PETRUCCI, T., POCCHIARI, F., SILANO, V., and AVELLA, R. 1971. Varietal differences in water-soluble gliadin fractions of *Triticum aestivum* and of *Triticum durum* seeds. *J. Sci. Food Agric.* 22:72-74.
- MINETTI, M., PETRUCCI, T., CATTANEO, S., POCCHIARI, F., and SILANO, V. 1973. Studies of the differential staining of wheat albumins, globulins and gliadins in polyacrylamide gel by aniline blue-black. *Cereal Chem.* 50:198-209.
- MITROFANOVA, O. P. 1976. Genetic control of the gliadin of soft wheat *T. aestivum* of the Chinese Spring variety. *Tsitol. Genet.* 10:244-247.
- MOCHIZUKI, A. 1968. The monosomics of durum wheat. Pages 310-315 in: *Proc. 3rd Int. Wheat Genet. Symp., Canberra*. K. W. Finlay and K. W. Shepherd, eds. *Aust. Acad. Sci.*
- MORRIS, R., and SEARS, E. R. 1967. The cytogenetics of wheat and wheat relatives. Pages 19-87 in: *Wheat and Wheat Improvement*. K. S. Quisenberry and L. P. Reitz, eds. *Am. Soc. Agron., Madison, WI.*

- MORRIS, R., SCHMIDT, J. W., MATTERN, P. J., and JOHNSON, V. A. 1966. Chromosomal locations of genes for flour quality in the wheat variety Cheyenne using substitution lines. *Crop Sci.* 6:119-122.
- MORRIS, R., SCHMIDT, J. W., MATTERN, P. J., and JOHNSON, V. A. 1973. Chromosomal locations of genes for high protein in the wheat cultivar Atlas 66. Pages 715-718 in: *Proc. 4th Int. Wheat Genet. Symp.*, Columbia, MO. E. R. Sears and L. M. S. Sears, eds. *Agr. Exp. Stn., Univ. of Missouri, Columbia.*
- MORRIS, R., MATTERN, P. J., SCHMIDT, J. W., and JOHNSON, V. A. 1978. Studies on protein, lysine, and leaf rust reactions in the wheat cultivar Atlas 66 using chromosome substitutions. Pages 447-454 in: *Proc. 5th Int. Wheat Genet. Symp.*, New Delhi, India. S. Ramanujam, ed. *Indian Soc. Genetics and Plant Breeding, Ind. Agr. Res. Inst., New Delhi.*
- NAKANISHI, T., YOSHIZUMI, H., TAHARA, S., HAKURA, A., and TOYOSHIMA, K. 1979. Cytotoxicity of purothionin-A on various animal cells. *Gann* 70:323-326.
- NIMMO, C. C., O'SULLIVAN, M. T., and BERNARDIN, J. E. 1968. The relation of a "globulin" component of wheat flour to purothionin. *Cereal Chem.* 45:28-36.
- NODA, K., and TSUNEWAKI, K. 1972. Analysis of seed proteins in ditelosomes of common wheat. *Jpn. J. Genet.* 47:315-318.
- NORONHA-WAGNER, M., and MELLO-SAMPAYO, T. 1966. Aneuploids in durum wheat. In: *Proc. 2nd Int. Wheat Genet. Symp.*, Lund. J. Mackey, ed. *Hereditas, Suppl.* 2:382-392.
- O'FARRELL, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007-4021.
- OHTANI, S., OKADA, T., KAGAMIYAMA, H., and YOSHIZUMI, H. 1975. The amino acid sequence of purothionin A, a lethal toxic protein for brewers' yeasts from wheat. *Agric. Biol. Chem.* 39:2269-2270.
- OHTANI, S., OKADA, T., YOSHIZUMI, H., and KAGAMIYAMA, H. 1977. Complete primary structures of two subunits of purothionin A, a lethal protein for brewers' yeast from wheat flour. *J. Biochem.* 82:753-767.
- OKAMOTO, M. 1957. Asynaptic effect of chromosome V. *Wheat Inf. Serv.* 5:6.
- ORTH, R. A., and BUSHUK, W. 1973a. Studies of glutenin. I. Comparison of preparative methods. *Cereal Chem.* 50:106-114.
- ORTH, R. A., and BUSHUK, W. 1973b. Studies of glutenin. III. Identification of subunits coded by the D-genome and their relation to breadmaking quality. *Cereal Chem.* 50:680-687.
- ORTH, R. A., and BUSHUK, W. 1974. Studies of glutenin. VI. Chromosomal location of genes encoding for subunits of glutenin of common wheat. *Cereal Chem.* 51:118-126.
- OSBORNE, T. B. 1907. *The proteins of the wheat kernel.* Carnegie Inst. Washington, DC.
- OSBORNE, T. B. 1924. *The vegetable proteins.* Longmans Green and Co: London.
- OZAKI, Y., WADA, K., HASE, T., MATSUBARA, H., NAKANISHI, T., and YOSHIZUMI, H. 1980. Amino acid sequence of a purothionin homolog from barley flour. *J. Biochem.* 87:549-555.
- PACE, W., PARLAMENTI, R., RAB, A., SILANO, V., and VITTOZZI, L. 1978. Protein α -amylase inhibitors from wheat flour. *Cereal Chem.* 55:244-254.
- PAYNE, P. I., and CORFIELD, K. G. 1979. Subunit composition of wheat glutenin proteins isolated by gel filtration in a dissociating medium. *Planta* 145:83-88.
- PAYNE, P. I., LAW, C. N., and MUDD, E. E. 1980. Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. *Theor. Appl. Genet.* 58:113-120.
- PENCE, J. W., WEINSTEIN, N. F., and MECHAM, D. K. 1954. A method for the quantitative determination of albumins and globulins in wheat flour. *Cereal Chem.* 31:29-37.
- PETRUCCI, T., TOMASI, M., CANTAGALLI, P., and SILANO, V. 1974. Comparison of wheat albumin inhibitors of α -amylase and trypsin. *Phytochemistry* 13:2487-2495.
- PETRUCCI, T., SANNIA, G., PARLAMENTI, R., and SILANO, V. 1978. Structural studies of wheat monomeric and dimeric protein inhibitors of α -amylase. *Biochem. J.* 173:229-235.
- POMERANZ, Y. 1971. Composition and functionality of wheat-flour components. Pages 585-674 in: *Wheat Chemistry and Technology.* Y. Pomeranz, ed. *Am. Assoc. Cereal Chem. St. Paul, MN.*
- POPERELYA, F. A., and BABAYANTS, L. T. 1978. The GLD1B3 gliadin component group as a marker for the gene responsible for the resistance of wheat plants to stem rust. *Nauk. Im. V.I. Lenina* 6:6-8.
- PRADA, J., SANCHEZ-MONGE, R., SALCEDO, G., and ARAGONCILLO, C. 1982. Isolation of the major low molecular weight gliadins from wheat. *Plant Sci. Lett. In press.*

- QUINN, C. J., and DRISCOLL, C. J. 1967. Relationships of the chromosomes of common wheat and related genera. *Crop Sci.* 7:74-75.
- REDMAN, D. G. 1976. Structural studies on wheat (*Triticum aestivum*) proteins lacking phenylalanine and histidine residues. *Biochem. J.* 155:193-200.
- REDMAN, D. G., and EWART, J. A. D. 1973. Characterization of three wheat proteins found in chloroform-methanol extracts of flour. *J. Sci. Food Agric.* 24:629-636.
- REDMAN, D. G., and FISHER, N. 1968. Fractionation and comparison of purothionin and globulin components of wheat. *J. Sci. Food Agric.* 19:651-655.
- REDMAN, D. G., and FISHER, N. 1969. Purothionin analogues from barley flour. *J. Sci. Food Agric.* 20:427-432.
- RILEY, R. 1960. The meiotic behavior, fertility and stability of wheat-rye chromosome addition lines. *Heredity* 14:89-100.
- RILEY, R., and CHAPMAN, V. 1958a. The production and phenotypes of wheat-rye chromosome addition lines. *Heredity* 12:301-315.
- RILEY, R., and CHAPMAN, V. 1958b. Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature* 182:713-715.
- RILEY, R., COUCOLI, H., and CHAPMAN, V. 1967. Chromosomal interchanges and the phylogeny of wheat. *Heredity* 22:233-248.
- RODRIGUEZ-LOPERENA, M. A., ARAGONCILLO, C., CARBONERO, P., and GARCIA-OLMEDO, F. 1975a. Heterogeneity of wheat endosperm proteolipids (CM Proteins). *Phytochemistry* 14:1219-1223.
- RODRIGUEZ-LOPERENA, M. A., ARAGONCILLO, C., TORRES, J. V., CARBONERO, P., and GARCIA-OLMEDO, F. 1975b. Biochemical evidence of chromosome homoeology among related plant genera. *Plant Sci. Lett.* 5:387-393.
- RYBALKO, A. I. 1975. Hybridization and monosomic analyses of gliadins. *Dis. W. VSGI, Odessa.* 26 pp.
- SALCEDO, G., RODRIGUEZ-LOPERENA, M. A., and ARAGONCILLO, C. 1978a. Relationships among low MW hydrophobic proteins from wheat endosperm. *Phytochemistry* 17:1491-1494.
- SALCEDO, G., ARAGONCILLO, A., RODRIGUEZ-LOPERENA, M. A., CARBONERO, P., and GARCIA-OLMEDO, F. 1978b. Differential allelic expression at a locus encoding an endosperm protein in tetraploid wheat (*T. turgidum*). *Genetics* 89:147-156.
- SALCEDO, G., PRADA, J., and ARAGONCILLO, C. 1979. Low molecular weight gliadinlike proteins from wheat endosperm. *Phytochemistry* 18:725-727.
- SALCEDO, G., PRADA, J., SANCHEZ-MONGE, R., and ARAGONCILLO, C. 1980a. Aneuploid analysis of low molecular weight gliadins from wheat. *Theor. Appl. Genet.* 56:65-69.
- SALCEDO, G., SANCHEZ-MONGE, R., ARGAMENTERIA, A., and ARAGONCILLO, C. 1980b. The A-hordeins as a group of salt soluble hydrophobic proteins. *Plant Sci. Lett.* 19:109-119.
- SAMUELSSON, G. 1973. Mistletoe toxins. *Syst. Zool.* 22:566-569.
- SAMUELSSON, G., and PETTERSSON, B. M. 1971. The amino acid sequence of viscotoxin B from the European mistletoe (*Viscum album* (L.) Loranthaceae). *Eur. J. Biochem.* 21:86-89.
- SANCHEZ-MONGE, R., DELIBES, A., HERNANDEZ-LUCAS, C., CARBONERO, P., and GARCIA-OLMEDO, F. 1979. Homoeologous chromosomal location of the genes encoding thionins in wheat and rye. *Theor. Appl. Genet.* 54:61-63.
- SASEK, A., and KÖSNER, J. 1977. The localization of chromosomes affecting the gliadin component of the Kavkaz variety by means of monosomic analysis. *Cereal Res. Comm.* 5:215-223.
- SEARS, E. R. 1953. Nullisomic analysis in common wheat. *Am. Nat.* 87:245-252.
- SEARS, E. R. 1954. The aneuploids of common wheat. *Mo. Agric. Exp. Stn. Res. Bull., Columbia.* 572:1-58.
- SEARS, E. R. 1959. The aneuploids of common wheat. Pages 221-229 in: *Proc. 1st Int. Wheat Genet. Symp., Winnipeg.* B. C. Jenkins, ed. Univ. of Manitoba.
- SEARS, E. R. 1965. Nullisomic-tetrasomic combinations in hexaploid wheats. In: *Chromosome Manipulations and Plant Genetics.* R. Riley and K. R. Lewis, eds. *Heredity Suppl.* 20:29-45.
- SEARS, E. R. 1966. Chromosome mapping with the aid of telocentrics. In: *Proc. 2nd Int. Wheat Genet. Symp., Lund.* J. Mackey, ed. *Hereditas Suppl.* 2:370-381.
- SEARS, E. R. 1969. Wheat cytogenetics. *Annu. Rev. Genet.* 3:451-468.
- SEARS, E. R. 1972. *Agropyron*-wheat transfers through homoeologous pairing. (Abstr.) *Can. J. Genet. Cytol.* 14:736.
- SEARS, E. R. 1973. *Agropyron*-wheat transfers induced by homoeologous pairing. Pages 191-199 in: *Proc. 4th Int. Wheat Genet. Symp., Columbia, MO.* E. R. Sears and L. M. S. Sears, eds. *Agric. Exp. Stn., Univ. of Missouri, Columbia.*

- SEARS, E. R. 1974. Telocentric chromosomes in wheat and their uses. *Genetics* 77:59.
- SEARS, E. R. 1975. The wheats and their relatives. Pages 59-91 in: *Handbook of Genetics*. Vol. 2. R. C. King, ed. Plenum Press: New York.
- SEARS, E. R. 1977a. The origin and future of wheat. Pages 193-196 in: *Crop Resources*. D. Seigler, ed. Academic Press, Inc.: New York.
- SEARS, E. R. 1977b. Analysis of wheat-*Agropyron* recombinant chromosomes. Pages 63-72 in: *Interspecific Hybridization in Plant Breeding*. E. Sanchez-Monge and F. García-Olmedo, eds. Proc. 8th Eucarpia Congress, Madrid.
- SEARS, E. R., and OKAMOTO, M. 1958. Intergenomic chromosome relationships in hexaploid wheat. In: *Proc. 10th Int. Congr. Genet.* 2:258-259.
- SHEARER, G., PATEY, A. L., and McWEENY, D. J. 1975. Wheat flour proteins: The selectivity of solvents and the stability of gliadin and glutenin fractions of stored flours. *J. Sci. Food Agric.* 26:337-344.
- SHEPHERD, K. W. 1968. Chromosomal control of endosperm proteins in wheat and rye. Pages 86-96 in: *Proc. 3rd Int. Wheat Genet. Symp.*, Canberra. K. W. Finlay and K. W. Shepherd, eds. *Aust. Acad. Sci.*
- SHEPHERD, K. W. 1973. Homoeology of wheat and alien chromosomes controlling endosperm protein phenotypes. Pages 745-760 in: *Proc. 4th Int. Wheat Genet. Symp.*, Columbia, MO. E. R. Sears and L. M. S. Sears, eds. *Agric. Exp. Stn., Univ. of Missouri, Columbia.*
- SHEWRY, P. R., AUTRAN, J.-C., NIMMO, C. C., LEW, E. J.-L., and KASARDA, D. D. 1980. N-terminal amino acid sequence homology of storage protein components from barley and a diploid wheat. *Nature* 286:520-521.
- SILANO, V., FURIA, M., GIANFREDA, L., MACRI, A., PALESCONDOLO, R., RAB, A., SCARDI, V., STELLA, E., and VALFRE, F. 1975. Inhibition of amylases from different origins by albumins from the wheat kernel. *Biochim. Biophys. Acta* 391:170-178.
- SOLARI, R. M., and FAVRET, E. A. 1967. Linkage of genes regulating the protein constitution of wheat endosperm. *Wheat Newsl.* 14:19.
- SOLARI, R. M., and FAVRET, E. A. 1968. Genetic control of protein constitution in wheat endosperm and its implication on induced mutagenesis. Pages 219-231 in: *Mutations in Plant Breeding*. IAEA Proc. Series STI/PUB/182. Vienna.
- SOLARI, R. M., and FAVRET, E. A. 1970. Chromosome localization of genes for protein synthesis in wheat endosperm. *Biol. Genet. Inst. Fitotec. Castelar* 7:23-26.
- SOZINOV, A. A., STELMAKH, A. F., and RYBALKO, A. J. 1978. Genetic analysis of gliadins in common wheat varieties. *Genetika* 14:1955-1967.
- STUART, L. S., and HARRIS, T. H. 1942. Bactericidal and fungicidal properties of a crystalline protein isolated from unbleached wheat flour. *Cereal Chem.* 19:288-300.
- VITTOZZI, L., and SILANO, V. 1976. The phylogensis of protein α -amylase inhibitors from wheat seed and the speciation of polyploid wheats. *Theor. Appl. Genet.* 48:279-284.
- WAINES, J. G. 1969. Ph.D. thesis, Univ. of California. *Diss. Abstr.* pp. 69-79, 131.
- WAINES, J. G. 1973. Chromosomal location of genes controlling endosperm protein production in *Triticum aestivum* cv. Chinese Spring. Pages 873-877 in: *Proc. 4th Int. Wheat Genet. Symp.*, Columbia, MO. E. R. Sears and L. M. S. Sears, eds. *Agric. Exp. Stn., Univ. of Missouri, Columbia.*
- WARCHALEWSKI, J. R. 1977a. Isolation and purification of native alpha-amylase inhibitors from winter wheat. *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* 25:725-729.
- WARCHALEWSKI, J. R. 1977b. Isolation and purification of native alpha-amylase inhibitors from malted winter wheat. *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* 25:731-735.
- WOYCHICK, J. H., BOUNDY, J. A., and DIMLER, R. J. 1961. Starch gel electrophoresis of wheat gluten protein with concentrated urea. *Arch. Biochem. Biophys.* 94:477-481.
- WRIGLEY, C. W. 1970. Protein mapping by combined gel electrofocusing and electrophoresis: Application to the study of genotypic variations in wheat gliadins. *Biochem. Genet.* 4:509-516.
- WRIGLEY, C. W. 1976. Isoelectric focusing of seed proteins. Pages 211-264 in: *Biological and Biomedical Applications of Isoelectric Focusing*. N. Catsimpoolas and J. W. Drysdale, eds. Plenum Press: New York.
- WRIGLEY, C. W., and SHEPHERD, K. W. 1973. Electrofocusing of grain proteins from wheat genotypes. *Ann. N.Y. Acad. Sci.* 209:154-162.