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Strategies for the Improvement of Wheat-Grain Quality using Molecular Genetics

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Introduction

The weight of wheat grain harvested each year is greater than that of any other crop. The annual world production usually exceeds 300×10^6 metric tons (Harlan and Starks, 1980). Some is fed to animals or used as seed for the following year's crop but much is processed in the home or by industry into a wide range of food products. In Europe and nations of European descent, bread wheat (*Triticum aestivum*, a hexaploid) is processed into leavened bread, biscuits, cakes, pastries and breakfast cereals. In India most wheat is converted into chapatis, unleavened flat breads, whereas in the far East, noodles are the major product. Durum wheat (*Triticum durum*, a tetraploid) is processed into pasta products such as spaghetti, macaroni and lasagna in Europe and as couscous in Northern and Western Africa. For all these products there are three stages in manufacture: milling (i.e. grinding), processing and cooking. Different properties in the wheat grains are exploited at each stage of manufacture and these properties differ widely for the different food products. Consequently, there is an ever-continuing need to develop wheat cultivars with grain characteristics which will be recognized as improvements by the processor.

In this article we wish to illustrate the development of a strategy based on the manipulation of defined genes to produce varieties with improved bread-making properties for growing in the UK. To initiate this strategy it was first necessary to understand the genetic basis of important characters influencing milling and flour quality and define the gene products. Lipids, proteins and several other constituents of the grain have a major influence on wheat-flour properties but, because proteins are the direct product of genes, it has been easier to develop genetic manipulation strategies for them. This article will therefore focus on flour improvement via protein variation. Particular proteins which affect flour quality have been identified by genetic analysis and their genes mapped on chromosomes. 'Good' wheat-flour proteins have been

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distinguished from 'poor' proteins coded by variants of the same gene at the same chromosomal locus. New alleles producing proteins with improved structural characteristics are being sought in wheats and close relatives, collected from all over the world, and are being incorporated into wheat by existing breeding procedures. Genes representative of the important proteins have also been isolated using recombinant DNA techniques in readiness to insert directly into wheat chromosomes. When this approach is developed, the gene sequences can be modified before insertion to optimize the protein structure required to improve the flour. This theme of flour improvement is illustrated in *Figure 1* and is developed in this article.

The basis of flour quality

Wheats can have endosperms with either hard or soft texture, while doughs from different wheats can vary from those with a high resistance to stretching and low extensibility, to those with low resistance and high extensibility. Hard and soft milling wheats are not synonymous with strong and weak flours.

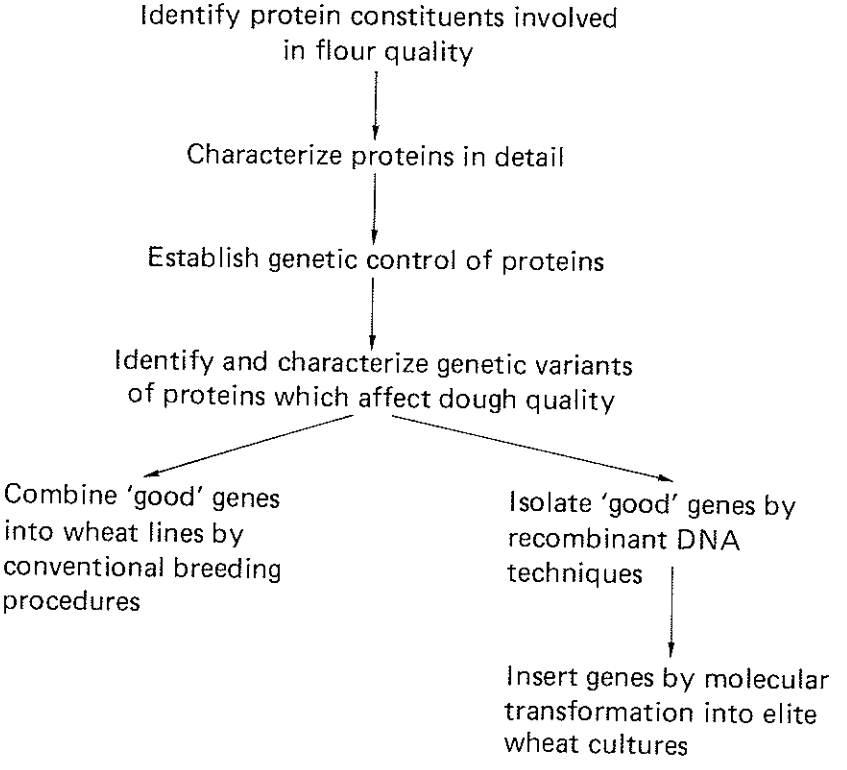

Figure 1. The strategy for manipulating wheat storage-protein genes.

HARD AND SOFT WHEATS

In the milling of soft-textured wheats, fractures occur across the dehydrated protoplasm. Fine, irregular particles are formed which have poor flow properties. On milling a hard-textured wheat, the endosperm protoplasm resists fracturing because of the strong adhesion between protein and starch: here, therefore, cleavage occurs mainly along the lines of the cell walls to produce large, regular-shaped particles which flow easily in bulk. Because in hard wheats the starch granules are rigidly fixed in the protein matrix they damage easily during grinding. This enables them to absorb much more water when wetted and their capacity to do this is double that of a soft-milling flour. For bread-making, the hard milling characteristic is of help in that the higher level of starch damage somewhat increases the yield of bread from a given weight of flour and lengthens the shelf life of bread before staling.

In practice, the wheat breeder has little difficulty in selecting for hard or soft milling types, firstly because this character is influenced much more by genotype than the environment and secondly because it is simply inherited, the controlling gene, *Ha*, occurring on chromosome 5D (Law *et al.*, 1978).

STRONG AND WEAK WHEAT

The breeding of wheats with strong dough characteristics is, however, not as straightforward as the development of hard or soft milling varieties. When wheat flour is mixed with water to form a dough it exhibits unique, visco-elastic properties. As the dough is mixed, its elastic properties increase to reach a peak and then begin to decline as mixing continues. The chemical and biophysical processes involved are complex and are only partly understood (Hoseney and Finney, 1974; Paredes-Lopez and Bushuk, 1983).

Biscuits are manufactured from weak wheats. High extensibility and very low elasticity ensures that biscuit shapes cut from dough sheets do not change in shape and thickness, which is essential both for appearance and for packaging. By contrast, bread doughs must be highly elastic as well as possessing some extensibility so that gases are retained during fermentation and a controlled expansion of the dough occurs. Between these two extreme uses for strong and weak wheats, wheats of a whole range of intermediate strengths are required for different products, for instance chapatis and Chinese and Japanese noodles.

The viscoelastic properties of wheat are principally governed by protein type and amount and it is these two variables which determine the mixing properties of the flour and the quality of the bread produced. Within limits, a high protein content can offset mediocre protein quality and vice versa (Nagl, 1982). Strong wheats are traditionally grown in continental climates of North America and Australia where a short growing season giving moderate yields guarantees a high protein content. In the maritime climate of Western Europe, wheat yields are high but protein content is low. Unfortunately, the amount of protein in a grain is determined much more by the environment than by the genetic make-up of the wheat plant so the development of new high-yielding cultivars with increased protein content is a difficult, although not impossible, task for

the wheat breeder to achieve. The improvement of wheat quality by altering the *kinds* of protein is, however, an alternative. To initiate this it was necessary to investigate the kinds of proteins in the grains of different wheat cultivars. These proteins are described in the next section.

Classification and characterization of the major wheat-grain proteins

Most proteins in the mature endosperm are deposited in protein bodies during grain development. These, the storage proteins, are prolamins which are soluble in certain aqueous alcohols and insoluble in salt solutions. The other major storage proteins in seeds and grains of flowering plants, the globulins, have the reverse of these solubility properties.

The proteins of wheat are usually classified into two major subgroups, glutenins and gliadins (Kasarda, Bernardin and Nimmo, 1976), and proteins of each group occur in approximately equal amounts in the endosperm. The two protein groups have different biochemical and biophysical properties. Glutenin occurs as large heterogeneous aggregates (molecular weight range by gel-filtration chromatography = 1×10^6 to $20–40 \times 10^6$) under dissociating but non-reducing conditions (Huebner and Wall, 1976) whereas gliadin occurs as a complex mixture of simple polypeptides (approximate molecular weight range by SDS-PAGE = 35 000 – 70 000). Upon the addition of a reducing agent, the chemical structure of gliadin remains similar but glutenin breaks down to its component subunits. About 80% of the subunits have molecular weights by SDS-PAGE of 35 000 – 50 000 and are called low-molecular-weight (LMW) subunits (Jackson, Holt and Payne, 1983) whereas the remaining subunits are larger, 90 000 – 14 000, and therefore are termed high-molecular-weight (HMW) subunits (Payne *et al.*, 1982).

The great complexity of glutenin subunits and gliadin is revealed by two-dimensional (2D) fractionation of grain proteins as illustrated for the variety Chinese Spring in *Figure 2*. In spite of this complexity, the three groups of protein monomers, gliadins, HMW glutenin subunits and LMW glutenin subunits, occur as discrete entities in the 2D map. The gliadins, when fractionated by electrophoresis at pH 3·2, can be classified into groups (α, β, γ and ω) on the basis of their mobilities (not shown). When a different variety is analysed by this procedure, then a similar complexity of subunits is obtained, but there can be extensive intervarietal variation in the exact position of individual components.

Information on the relationships between the different polypeptides and on their structures has come from studying cloned gene sequences. A cDNA clone bank was constructed to mRNA harvested from developing wheat endosperms (Bartels and Thompson, 1983). The bank was screened with radioactively labelled endosperm poly A^+RNA and clones giving a clear signal were selected for investigation of their coding properties. More than 90% of these clones coded for gliadin or HMW glutenin polypeptides, the ratio between the two groups being about 10 to 1.

The gliadin-encoding clones were further classified according to which family of the polypeptides they encoded. Generally, one clone hybridized to mRNA

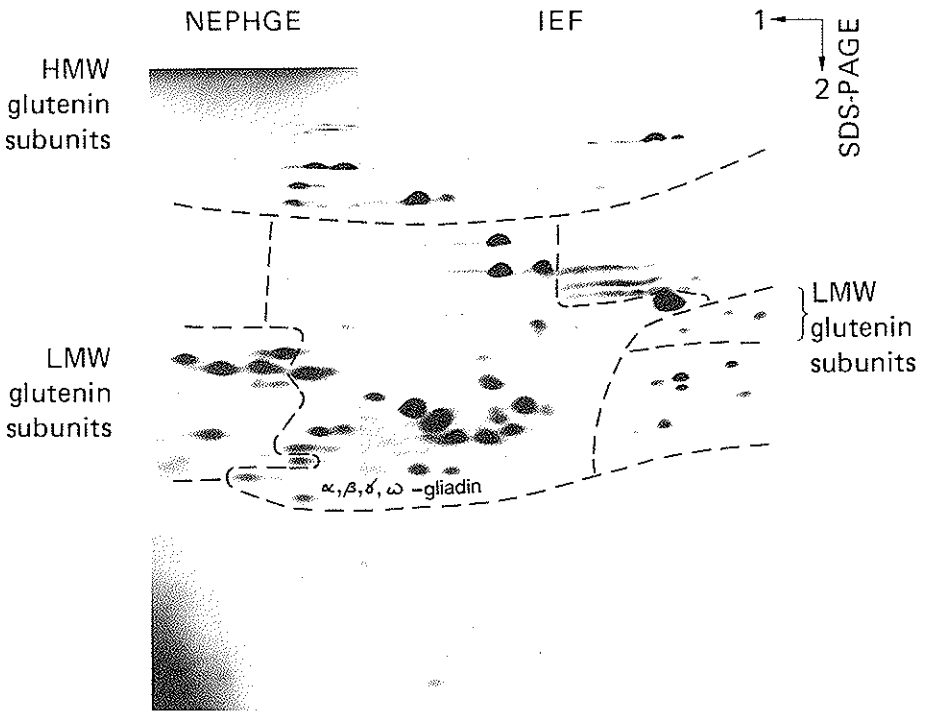

Figure 2. Fractionation of the total endosperm proteins of the variety Chinese Spring by two different two-dimensional electrophoresis systems. The distinction between glutenin subunits and gliadins was determined by gel filtration chromatography. NEPHGE is short for non-equilibrium pH gradient electrophoresis and IEF for isoelectric focusing. The method is described in Jackson, Holt and Payne (1983).

for more than one gliadin polypeptide, and in some cases the polypeptides belonged to different gliadin mobility groups. This result indicates the existence of sequence homology between gliadins of a large molecular weight range, for example, between the α and β gliadin protein families and those encoded by the *Gli-2* locus (*see later*). When sequence-related mRNAs are translated *in vitro* and the protein products analysed as in *Figure 2*, proteins varying in size and charge are observed. Thus many of the grain proteins in *Figure 2* are specified by sequence-related genes.

Knowledge of the primary sequences of the gliadin proteins is at an early stage but when the data from sequencing of the cDNA clones are put together with those for the closely related prolamins of barley (Matthews and Miflin, 1980; Forde *et al.*, 1981; Donovan, Lee and Longhurst, 1982; Rasmussen, Hopp and Brandt, 1983) the structure shown in *Figure 3a* emerges. The variation in structure between the various gliadin genes within a clone-defined family has yet to be defined but some of it may result from variation in the number of subrepeats found in region B of *Figure 3a*. These subrepeats would be expected to be relatively unstable and may give rise to deletions and

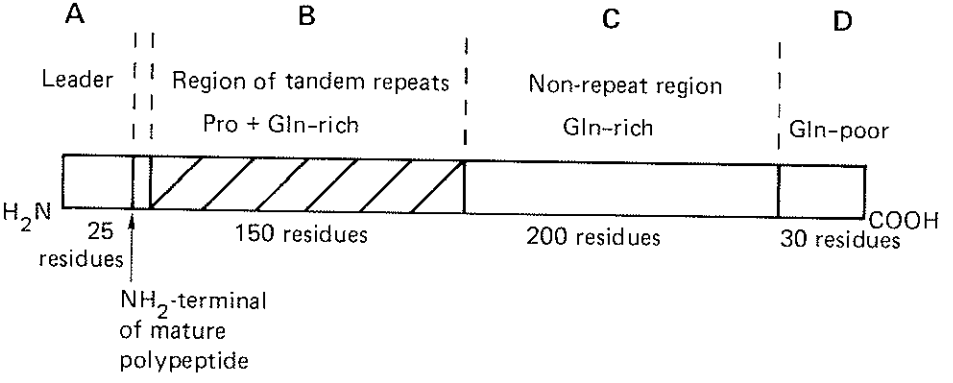

Figure 3a. Schematic representation of a typical gliadin polypeptide sequence. The lengths for each region and especially B and C vary considerably between different gliadin subgroups. $-NH_2$ = amino-terminal end of nascent chain; $-COOH$ = carboxyterminal end; Pro = Proline; Gln = Glutamine.

duplications to generate the observed length heterogeneity within gliadin subgroups and between alleles.

No complete glutenin primary sequence has yet been elucidated. The HMW subunit sequences appear to be as illustrated in *Figure 3b*. This has been deduced from sequencing the amino-terminal end of the protein (Shewry *et al.*,

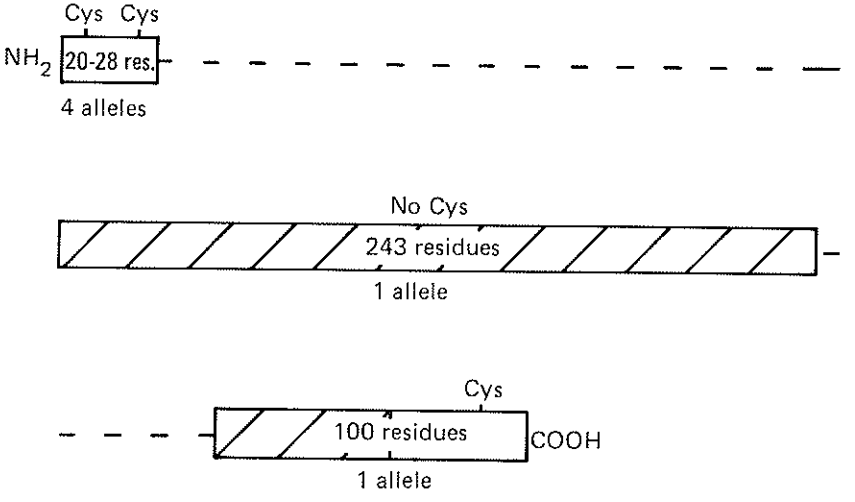

Figure 3b. Schematic representation of a typical HMW glutenin polypeptide sequence. The total polypeptide length is estimated to be between 570 and 700 residues, of which 20–28 amino-acid residues have been determined by protein sequencing at the amino (NH_2) terminal and 100 amino acid residues from DNA sequencing, at the carboxyl (COOH) terminal, and a 243 amino-acid stretch internally. (The position of the internal sequence relative to the termini has not yet been determined.) The protein sequence has been determined for four different alleles, whereas the DNA sequence was determined for individual cDNA clones. Cys = cysteine.

1984) and from the analysis of cDNA clones derived from nearer the carboxyl end (Forde *et al.*, 1983; Thompson *et al.*, 1983). The DNA clones specifying the gliadins do not show homology with those specifying HMW glutenin subunits, showing that the proteins differ considerably in sequence.

Before genetic manipulation of the genes specifying these proteins could be approached in a direct way to improve dough quality, it was necessary to discover the genetic basis for the protein families. The results of these analyses are described in the next section.

The genetic organization of gliadin and glutenin genes

Bread wheat is hexaploid and is made up of three related diploid genomes termed A, B and D. Many genetic stocks are available which have chromosome deletions or deficiencies (Sears, 1954), or substitutions where single chromosomes from one cultivar are substituted into another (Law and Worland, 1973). Analysis of these stocks by electrophoresis has enabled the chromosomal location of the genes controlling the synthesis of individual glutenin subunits and gliadin polypeptides to be determined (Payne *et al.*, 1982).

The conclusions reached from such analyses (*Table 1*) show that only two homoeologous chromosome series contain genes which control storage protein synthesis; the HMW subunits of glutenin are controlled by genes at *Glu-1* loci on the long arms of chromosomes 1A, 1B and 1D whereas the LMW subunits are controlled by genes at *Gli-1* loci on the short arms of the same chromosomes. Some of the gliadin proteins (ω- and γ-gliadins) are also controlled by genes at the *Gli-1* loci whereas the rest (the α- and β-gliadins) are located at *Gli-2* loci on chromosomes 6AS, 6BS and 6DS. These results have been confirmed by hybridization of the gliadin and glutenin cDNA clones to DNAs extracted from euploid plants and from the plants lacking individual chromosomes. DNA sequences specifying most of the α- and β-gliadins reside only on the short arm of chromosomes 6A, 6B and 6D while those defining the gliadins which include the γ- and ω-gliadins are only on group 1 chromosomes (N.P. Harberd, unpublished work).

Table 1. Chromosomal location of the storage-protein genes of the wheat endosperm

Gene locus	Chromosome	Arm	Position	Storage proteins coded for
Glu-A1	1A	Long	Close to centromere	HMW subunits of glutenin
Glu-B1	1B	Long	Close to centromere	
Glu-D1	1D	Long	Close to centromere	
Gli-A1	1A	Short	Towards the end	ω-gliadins γ-gliadins and LMW glutenin subunits
Gli-B1	1B	Short	Towards the end*	
Gli-D1	1D	Short	Towards the end	
Gli-A2	6A	Short	Towards the end	α-gliadins β-gliadins
Gli-B2	6B	Short	Towards the end*	
Gli-D2	6D	Short	Towards the end	

* The genes on these chromosomes are located on the short arm satellites (the terminal part of the chromosome arm beyond the nucleolar organizing region).

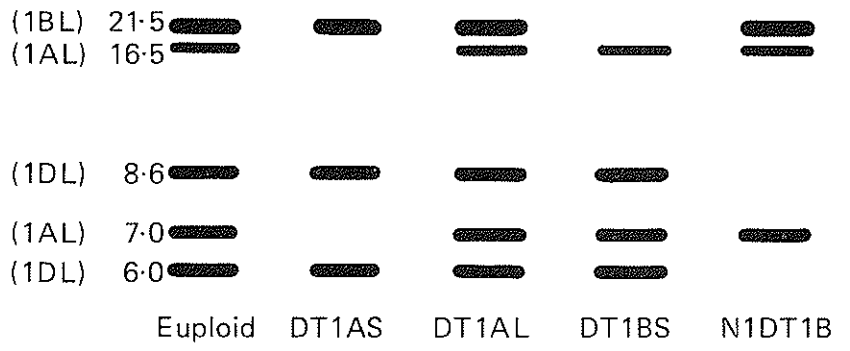

Figure 4. Hybridization of radioactively labelled HMW glutenin subunit cDNA probe to *Eco* RI restriction enzyme digests of DNA from euploid wheat DNA (variety Chinese Spring) and genetic stocks of this variety lacking the long arm of chromosome 1A (DT1AS), the short arm of 1A (DT1AL), the long arm of 1B (DT1BS) or the complete chromosome 1D (N1DT1B). The DNA fragments were size-fractionated by electrophoresis (from top to bottom) on agarose gels and transferred to a nitrocellulose filter before hybridization. The sizes of the DNA fragments are given in kilobases, and their chromosomal location is given in brackets.

The cDNA clones specifying HMW glutenin subunits hybridized to similar extents to sequences only on the long arms of chromosomes 1A, 1B and 1D (*Figure 4*), showing that the homoeoallelic genes are clearly related and different from at least most gliadin genes. Using the restriction enzyme *Eco* RI, it was possible to distinguish the DNA fragments carrying the different glutenin subunit genes. Which restriction fragment corresponds to which locus was established by determining which fragment(s) are absent in DNAs isolated from plants lacking the long arms of chromosome 1A, 1B or 1D (*Figure 4*). It was estimated from the hybridization experiments that there are only 10–15 glutenin genes per hexaploid chromosome complement (Thompson *et al.*, 1983). In contrast, the number of gliadin gene sequences is considerably greater — at least 20 per clone-defined family in hexaploid wheat (N.P. Harberd, unpublished work). The genetic evidence, based on the number of proteins expressed by one chromosome locus, suggests that there are two genes at each of the triplicate *Glu-1* loci (i.e. 12 per hexaploid cell) but several at each of the *Gli* loci. Thus there is good agreement between the number of genes detected by molecular hybridization and the number of gene products detected by electrophoresis.

In further genetic experiments, progenies of various crosses whose parents had contrasting storage-protein variants were screened by electrophoresis. Recombination between storage protein genes within a locus was extremely rare and was detected only for HMW subunits of glutenin coded by chromosome 1B and at the frequency of 1 in 900 progeny. Therefore storage-protein genes must be tightly clustered, there being only one major locus containing these genes for each chromosome arm (Payne *et al.*, 1983). Thus, blocks of storage proteins are inherited together, so that glutenin and gliadin, although biochemically complex, are much simpler in their inheri-

tance. Analysis of many hundreds of cultivars and progeny of crosses indicates that each locus exhibits extensive allelic variation so that any one cultivar will have a particular combination of alleles for each of the nine storage protein loci (Sozinov and Poperelya, 1980; Payne, Holt and Law, 1981).

Recognition and characterization of protein variants affecting dough quality

The property of dough which is important in bread-making is its viscoelasticity. The physical and molecular basis of viscoelasticity is not known in detail. Elasticity (dough strength) is principally due to the long glutenin molecules, thought to be built up from HMW (and probably LMW) subunits being linked together, end to end by disulphide bonds (Ewart, 1977; for review *see* Miflin, Field and Shewry, 1983). The molecular weight of the aggregates (several millions) is likely to allow considerable elasticity. Protein quality for bread making appears to be directly, and primarily, related to the mean molecular weight of the glutenin aggregates (Huebner and Wall, 1976).

The protein sequence data currently available for HMW glutenin subunits are consistent with the hypothetical structure of glutenin (*Figure 5*). The amino-terminal sequences include two cysteine residues in the first 10 residues and the carboxyl-terminal sequences of two cDNA glutenin clones each contain a single cysteine (Forde *et al.*, 1983; Thompson *et al.*, 1983; Shewry *et al.*, 1984). The DNA sequencing of the two incomplete glutenin cDNAs has also recently revealed that the proteins contain long stretches of amino-acid residues built from simple repeat units. These repeat units contain glycine, glutamine and proline and so the high content of these amino acids in glutenin is explained by the repeat-unit structure (*Figure 3b*). Attributing the important property of elasticity to the HMW glutenin proteins does not imply that gliadins play no part in bread-making. Indeed, being viscous and imparting extensibility, they are very much involved in expansion of the dough during fermentation. However, in our view the intervarietal variation in dough strength is due to differences in the mean molecular weight of glutenin aggregates which in turn is due to structural variation in the different glutenin subunits. These structural differences may involve the number and distribution of cysteines or

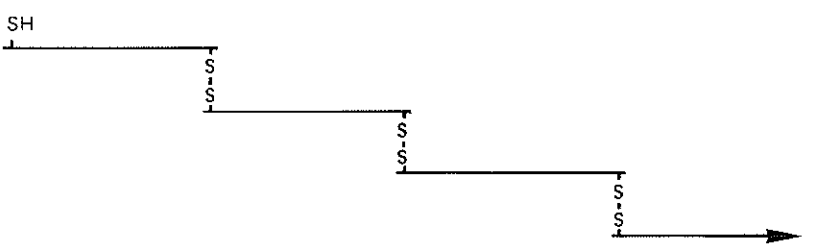

Figure 5. Model of HMW glutenin aggregation according to Ewart (1977). HMW glutenin polypeptide chains are linked close to their termini by disulphide cross-bridges (–S–S–) to produce inter-chain aggregates of molecular weights of several millions.

other amino-acid residues which influence the ability of these aggregates to interact with each other and also with gliadins and lipids. The repeating-unit structure revealed by DNA sequencing (*Figure 3b*) suggests that the length variants in individual glutenin subunits probably result from internal deletions or duplications. Such events are common in genes containing tandemly arranged repeat units.

To test the hypothesis that variation in HMW glutenin subunits is responsible for variation in dough quality between plants, cultivars with contrasting glutenin subunits and bread-making quality were crossed. F_3 grain, bulked from individual F_2 plants, was analysed by electrophoresis for protein type and by the SDS-sedimentation test for protein quality. Sedimentation volume of flour in SDS in this test is positively correlated with baking quality (Axford, McDermott and Redman, 1979). The progeny analysis is outlined in *Figure 6* for the cross between the varieties Alcedo and Brigand, the former having good bread-making quality and the latter, a biscuit wheat with poor bread quality. The mean sedimentation volume of those progeny which were homozygous for subunits 5 and 10, inherited from Alcedo and coded at *Glu-D1*, was appreciably higher than the progeny which were homozygous for their allelic counterparts, subunits 2 and 12, inherited from Brigand. Statistical analysis revealed that the difference was significant at $P < 0{\cdot}001$. About half the progeny fitted into one of the above two categories whereas the rest were heterozygous for subunits 2 and 12 and subunits 5 and 10. Their mean sedimentation volume was intermediate between those of 5/10 and 2/12. These two pairs of subunits have also been compared for quality in progeny from several different crosses and subunits 5 and 10 were on average superior to 2

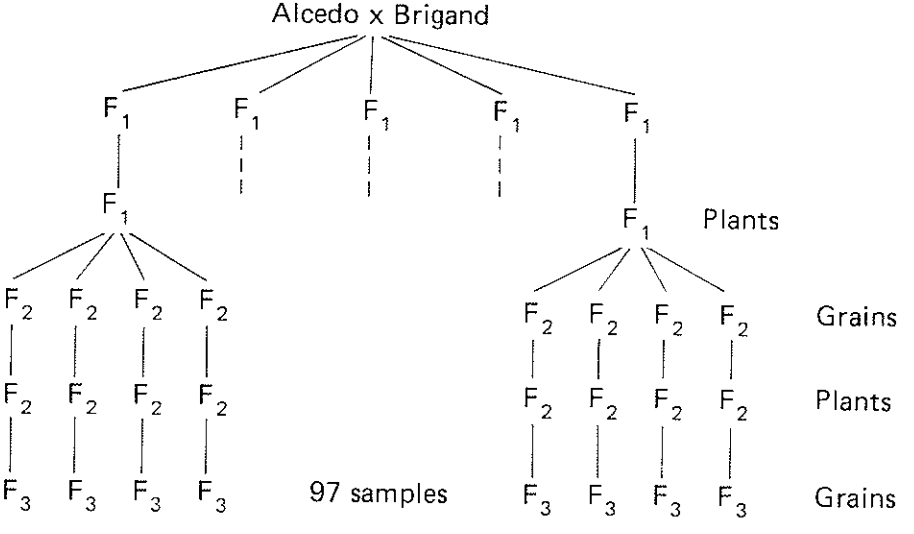

Figure 6. Quality testing of HMW subunits in progeny of Alcedo × Brigand. Each F_3 grain sample tested for: (1) SDS-sedimentation volume; (2) subunit composition. N.B. Progeny at each generation were selected at random.

and 12 in every experiment, although the level of significance did vary. Many crosses have now been analysed for many contrasting allelic pairs of subunits. For instance, for *Glu-D1* the order of decreasing quality associations is 5 and 10 > 2 and 12 = 3 and 12 > 4 and 12 (Payne *et al.*, 1983). Similar procedures have been used to demonstrate variation for quality at the *Gli-1* loci but the extent of variation is less than at *Glu-1*. Allelic variation for protein quality has also been reported for *Glu-1* by Moonen, Scheepstra and Graveland (1983) and for *Gli-1* by Sozinov and Poperelya (1980).

Preliminary indications are that the effects of 'good quality' alleles from different loci are predominantly additive for protein quality. To confirm and extend these results, near-isogenic lines are being developed in a single cultivar (Payne *et al.*, 1983). These have a similar genotype to the recipient except that a single allele at one storage protein locus has been replaced by another from a different cultivar. The near-isogenic lines will also be tested in field trials to determine if any 'good quality' alleles are tightly linked to genes which confer undesirable traits, such as poor yield.

When good-quality alleles are identified they can be purified by recombinant DNA techniques. For example, by hybridizing an HMW glutenin cDNA sequence to restriction-enzyme-digested DNA from the series of near-isogenic lines described above, it may be possible to distinguish the particular glutenin subunit gene responsible for the better protein, just as the different genes were distinguished in Chinese Spring (*Figure 4*). DNA from this plant will be cloned in bacteriophage λ and phage carrying glutenin subunit genes will be purified. The phage carrying the good-quality allele will be distinguished from phage carrying other HMW glutenin sequences by its particular distribution of restriction enzyme sites.

By sequencing the coding and control regions of the genes for good and poor alleles, the structural variation underlying variation in dough quality should be determined.

Incorporation of 'better' HMW glutenin genes in wheat-breeding programmes

BY ESTABLISHED BREEDING METHODS

In the previous section, several glutenin subunits were shown to be correlated with improved dough quality. Glutenin subunits are inherited as pairs of tightly linked genes; consequently, it is now possible to screen cultivars for these pairs of subunits and to recommend the appropriate combination to the wheat breeder, to allow him to create genotypes which should have combinations of good alleles at the *Glu-1* loci on chromosomes 1A, 1B and 1D. Such lines should, theoretically, make better dough than parental varieties with fewer good alleles.

Such recommendations have been put into practice in the breeding programmes at the Plant Breeding Institute, Cambridge. F_3 plants were screened in the field by the wheat breeder for good agronomic characters and disease resistance, the F_3 grain of the selected plants were tested for bread-making quality by the SDS-sedimentation test and about 10% of those

giving the highest quality values were analysed by SDS-PAGE. Selections were made for progeny which were doubly homozygous for quality subunits; progeny which were heterozygous for the quality subunits or homozygous for poor-quality subunits were rejected. The advantage of choosing quality homozygotes by this procedure is that in successive generations to the final selection at F_8 or later, no further screening by SDS-PAGE will be necessary. The usefulness of genetics and protein electrophoresis to the wheat breeder as described above is to reduce the time taken to obtain cultivars with improved bread-making quality.

The glutenin genes discussed so far have been found in adapted wheat varieties. However, electrophoretic analyses of glutenin proteins in land races of primitive agriculture have identified numerous subunits with electrophoretic mobilities different from any seen in modern cultivated wheat. Some of these are being backcrossed into adapted wheat to assess their usefulness for improving flour quality (Payne *et al.*, 1983). Other new glutenin genes have been found in *Triticum* and *Aegilops* species — diploids related to the progenitors of hexaploid wheat. Genes from these species can be introduced into wheat using special genetic approaches (Law and Payne, 1983). Recombination between the wild diploid chromosomes and those of hexaploid bread wheats is normally suppressed when hybrids are made. However, when chromosome 5B of wheat is deleted, recombination is enhanced, allowing transfer of chromosome segments of the wild diploid to hexaploid wheat chromosomes (Riley and Chapman, 1958). Thus a backcrossing programme to an adapted wheat is carried out after one or a few generations in the absence of chromosome 5B, to introduce the alien glutenin genes. Several such HMW glutenin subunit gene introductions are currently in progress.

BY INTRODUCTION OF PURIFIED GENES

In plant molecular biology, opportunities are emerging for the introduction of new genes into plant chromosomes. There are several reports of genes being introduced into dicotyledonous plants in such a way that they are expressed in the recipient plant (Bevan, Flavell and Chilton, 1983; Fraley *et al.*, 1983; Herrera-Estrella *et al.*, 1983a, b). Such a transformation system is not yet available for monocotyledonous species such as wheat. However, there is good reason to be optimistic that this will be developed over the next few years (Flavell and Mathias, 1984). Thus it is not too early to assess how the introduction of cloned genes for storage-protein genes might improve dough quality.

Firstly, if it becomes possible to insert HMW glutenin subunit genes in such a way that their expression is still well regulated during grain development, then the transfer of a series of alleles to a series of varieties will be more rapid than by conventional backcrossing procedures. Results from genetic analysis already performed suggest that because there are so few HMW glutenin genes, introduction of only one 'good' allele improves dough quality. Therefore the introduction of only one gene by molecular transformation without deletion of existing poor alleles should improve quality. Clearly, to achieve appropriate

expression of the gene it will be necessary to transfer a segment of DNA that includes large tracts of DNA on both sides of the coding sequence. Such tracts to the 5′ side will contain the TATA and NGAA boxes usually necessary for gene expression, and other sequences yet to be identified which regulate the timing and amount of transcription (Messing *et al.*, 1983).

Secondly, it will be possible to modify the gene structure before insertion to enhance dough quality. It may also be possible to manipulate the regulatory regions to boost transcription to a higher relative level than that of the genes at *Glu-1* loci. This would enhance the effects of the 'good' allele over the 'poorer' existing alleles.

Thirdly, it should be possible to modify the number of HMW glutenin genes in wheat. Genetic analyses have already shown that dough strength as measured by the SDS sedimentation test declines rapidly as the dosage of those proteins is decreased. Thus, some cultivars of world agriculture have a null allele for *Glu-A1* so that no HMW subunit of glutenin is produced from this locus. The null allele is associated with poor quality. Several land races of primitive agriculture have nulls at *Glu-B1* and *Glu-D1*, the other HMW glutenin subunit loci. These nulls are being combined and preliminary results have shown that they have a dramatic effect in decreasing quality (Payne *et al.*, 1984). From these results, it is likely that increasing the number of genes coding for HMW glutenin subunits will improve dough strength, provided that the total proportion of these subunits to total protein is also increased. Unfortunately, increasing gene dosage by classical methods is extremely difficult, but should be possible by molecular transformation techniques.

Fourthly, introduction of genes as single genetic units, rather than as chromosome segments as in conventional breeding procedures, will not generate any problems attributable to the close proximity of deleterious genes to the storage protein loci.

Manipulation of gliadin genes

FOR BAKING QUALITY

Much of the nitrogen assimilated into developing grains is incorporated into gliadins. If the amount of gliadin synthesis could be reduced, some of this nitrogen would be used to synthesize more HMW glutenin, so increasing the ratio of glutenin to gliadin. As spontaneous partial deletions occur for 1BS involving the loss of *Gli-B1* there is scope here for use of this approach. Mutants which have chromosome cleavages immediately proximal to *Gli-1* are likely to be most useful. Any genes which are distal to *Gli-1* will, of course, also be lost and the effect of this for practical plant breeding has yet to be assessed. There is therefore a need to assess the performance in field trials and baking tests of lines carrying deletions of the terminal segment of 1BS which probably has reduced gliadin synthesis.

In the several thousand analyses carried out so far, no land races or spontaneous mutants from genetic crosses have deleted *Gli-2* loci. Any means of specifically inactivating ω- or γ-gliadin genes at *Gli-1* without affecting the

genes for LMW glutenin subunits, or the inactivation of gliadins at *Gli-2* without affecting adjacent genes, might have useful implications for grain quality improvement.

A mutation in barley, which involves deletion of a large part of the *Hor-2* locus (Kreis *et al.*, 1983) (a complex locus specifying many hordeins, similar to the gliadins in wheat) was found after treatment of seed with γ-rays (Doll, 1976). Such an approach might therefore produce the desired deletions at the *Gli-1* or *Gli-2* loci. Another mutation in barley at the *Lys-3A* locus causes a reduction in hordein levels (Doll, 1976; Thompson and Bartels, 1983). If an equivalent mutation were available in wheat it would be interesting to assess its effect on quality, although such mutations may reduce yields and so make the varieties of little commercial value.

FOR NUTRITIONAL QUALITY

The amino-acid composition of wheat grains is not optimal for animal consumption. The concentration of some of the important amino acids, e.g. lysine, is too low (lysine constitutes less than 1% of the amino acids in the abundant gliadins (Miflin, Field and Shewry, 1983)). If the lysine content could be increased, then the grain would be more suitable for animal consumption, thus reducing the protein supplements currently required. When a gene has been isolated and sequenced it is possible to change specific codons and hence alter the amino-acid sequence and composition of its protein product. However, because the gliadin genes are present in high copy number, it is difficult to envisage inserting a single gliadin gene, modified in certain codons, to increase the proportion of lysine, and achieving a substantial change in the overall amino-acid balance of the grain proteins. However, it might be possible if several genes could be inserted with much more powerful promoters than the existing gliadin genes. It might also be easier to achieve if the starting genotype carried major deletions of gliadin genes or a mutation reducing their expression, as outlined above. Lysine is a positively charged amino acid which may adversely affect the physical structure and solubility properties of the new gliadin and its deposition in grain protein bodies. It might be necessary, therefore, to construct many genes with lysine codons at different positions to select the most favourable gene structure. Thus, although improving the nutritional quality of wheat grain protein is a difficult task, the tools of molecular biology offer new opportunities which are not possible using older-established techniques.

Concluding remarks

The strategies described in this article employ the techniques of protein electrophoresis and the isolation of genes by cloning in bacteria — both experimentally well developed. However, their application to solve problems of crop improvement is in its infancy. Indeed, the project outlined here is one of the first in plant breeding to reach the stage where biochemical and molecular techniques have been used to identify specific alleles, the presence of which

leads to crop improvement, and where those genes have been isolated using recombinant DNA techniques. We believe that the routes we have described to wheat-flour improvement will serve as a useful model for dissecting and manipulating other complex problems in plant and animal breeding.

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