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Genetic engineering of wheat gluten

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Wheat is the number one food crop in the world based on area under cultivation and total production. The popularity of wheat is based on diverse uses, resistance to many pathogens and pests, low cost of production, rapid growth, genetic flexibility and adaptability to different climates. The unique breadmaking quality is related to the type and quantity of gluten proteins, especially the high-molecular-weight glutenin subunits that are synthesized and stored in the seed endosperm. Recent advances in genetic transformation of wheat, including the integration and expression of high-molecular-weight glutenin subunit genes, now make it possible to engineer the gluten proteins in order to improve breadmaking qualities.

Primitive forms of wheat were among the first plants to be domesticated in the Neolithic age, nearly 10 000 years ago, followed by the hexaploid bread wheats (*Triticum aestivum*), which became abundant about 6000 BC. As a result of intensive breeding in modern times, aimed at improving adaptability to a wide range of ecological conditions (temperate, subtropical and tropical), wheat is now the most widely cultivated crop in the world. Even in Asia, where rice has historically been the dominant crop, wheat is well on the way to becoming the number one crop; for example, in 1994, Asia produced 217 million tons of wheat, compared with the combined harvest of 209 million tons in the USA, Canada, Europe and Mexico. The wheat grain, of which about 10% (dry weight) is protein, is a major source of energy and nutrition in the human diet. The majority of the seed proteins are stored in the starchy endosperm in the form of prolamins, which are unique to cereal grains, and account for over half of the total seed nitrogen. Prolamins in general are known for their nutritional qualities, but only in wheat are they associated with functional quality.

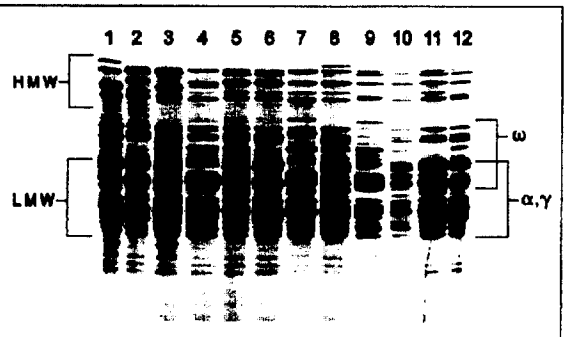

Fig. 1. Seed storage proteins of wheat. Total wheat seed proteins from a set of different bread wheat cultivars were extracted, separated using polyacrylamide gel electrophoresis and visualized with the stain Coomassie blue. Each lane of the gel (1–12) contains the protein from a different cultivar. Classes of wheat prolamin polypeptides indicated include: high-molecular-weight glutenin subunits (HMW); low-molecular-weight glutenin subunits (LMW); and α-, γ- and ω-gliadins. There is extreme polymorphism within the different protein classes. The HMW-glutenin subunits are well separated from the other prolamins and comprise 5–10% of the total protein. *Reproduced, with permission, from Ref. 30.*

The gluten proteins

During the mixing of wheat flour with water to make dough, the prolamins form the gluten, a continuous proteinaceous network that is the basis of dough functionality. The prolamins of wheat are highly polymorphic polypeptide mixtures of >50 components with M_r values ranging from 30 000 to 90 000 (Refs 1 and 2). They are characterized by high combined levels (40–75%) of glutamine and proline, and their unusually high solubility in water–alcohol solutions. The wheat prolamins are divided on the basis of function into two groups, the glutenins and gliadins, which together confer the properties of elasticity (strength) and extensibility (viscosity). These unique properties of wheat gluten are not found in the storage proteins of other cereals and are the basis of the wide range of food products derived from wheat. Figure 1 shows the seed protein patterns from 12 different wheat cultivars and illustrates the different classes of prolamins – they form a very heterogeneous group. The gliadins are monomeric molecules (30 000–75 000 kDa) divided into several classes (α-, γ- and ω-gliadins). In contrast, the glutenins form large polymeric structures as a result of intermolecular disulfide bonds. These gluten polymers, with M_r values >10 million, are some of the largest protein molecules found in nature[3]. The glutenins are divided into a low-molecular-weight glutenin subunit (LMW-GS) group and a high-molecular-weight glutenin subunit (HMW-GS) group. The HMW-GSs (65 000–90 000 kDa), which represent approximately 0.5% of the total seed dry weight, have been studied extensively because of their effect on elasticity, and hence the breadmaking quality of wheat dough.

The genetics of high-molecular-weight glutenin subunit genes

Bread wheat is a hexaploid species comprised of three different but related genomes, termed A, B and D. Each genome contributes seven pairs of chromosomes. Extensive genetic and molecular analyses of wheat lines and prolamin composition have established the chromosomal positions of the prolamin genes[1]. The HMW-GS genes (*Glu-1*) are located on the long arms of the homologous chromosomes 1A, 1B and 1D (Fig. 2). Genes encoding LMW-GS (*Gli-1*), γ-gliadins and ω-gliadins are grouped at loci on the short arms of chromosomes 1A, 1B and 1D. The genes for α-gliadins (*Gli-2*) are found on the short arms of chromosomes 6A, 6B and 6D.

The HMW-GSs represent 5–10% of the total seed protein, with each HMW-GS gene accounting for up to 2% of the

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total protein[1,4]. The HMW-GSs are further divided into high M_r x-type and low M_r y-type subunits, based on their electrophoretic mobility and isoelectric points (Fig. 1). Tightly linked pairs of one x-type and one y-type gene are present at each locus on the 1A, 1B and 1D chromosomes of hexaploid wheat[1] (Fig. 2). The encoded polypeptides are similar in structure and are assumed to have arisen by gene duplication in an ancestral grass. Six HMW-GS genes are present in each cultivar of hexaploid bread wheat, but because of gene silencing only three, four or five subunits are synthesized in different cultivars. The HMW-GSs 1Bx, 1Dx and 1Dy are present in all cultivars, 1By and 1Ax are present in some cultivars, but no cultivars contain 1Ay, because the gene is always silent. Similarly, the tetraploid durum wheats, with the A and B genomes, contain four HMW-GS genes, of which no more than three are active.

The different alleles of each gene encoding HMW-GSs are numbered according to a system proposed by Payne and Lawrence[5]. Thus, HMW-GS No. 1 is encoded by the *Glu-1A-1a* gene (in common usage, the Ax1 subunit from the *Ax1* gene). The HMW-GSs 1Ax1 and 1Ax2*, and the 1Dx5 + 1Dy10 subunit pair, are said to be associated with stronger doughs, and the allelic pair 1Dx2 + 1Dy12 with weaker doughs. Thirteen HMW-GS genes have been isolated and sequenced from bread wheats and ancestor genomes, including the entire set of six genes from a single high-quality cultivar[6], and pairs of alleles correlated with good and poor dough-processing characteristics[7] (all sequences are available through GenBank).

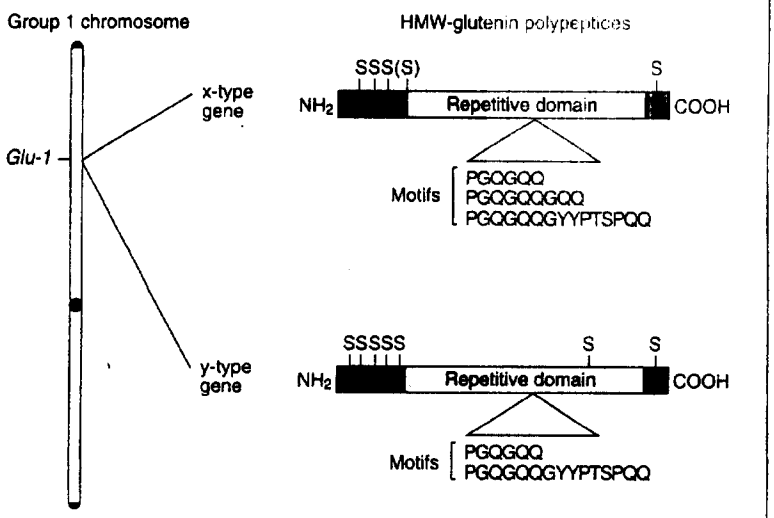


Fig. 2. Chromosomal location of the high-molecular-weight glutenin subunit (HMW-GS) genes and the structure of the encoded polypeptides. The HMW-GSs are encoded by genes at the *Glu-1* loci on the long arms of the group 1 chromosomes. Each locus contains an x- and a y-type HMW-GS gene. The two genes have never been confirmed to be separated in recombination experiments. The two HMW-GS types have a similar structure: a central, repetitive domain comprising repeats of six, nine and 15 amino acids; and smaller, terminal domains containing most of the cysteine residues (disulfide bonds are indicated by 'S'). Allelic variation in HMW-GS size is caused by deletions or duplications within the repetitive domain, caused by recombination events among the repeated DNA sequences. The x-type HMW-GSs apparently have one intramolecular disulfide bond within the N-terminus, and the y-type is likely to have two such bonds, thus reducing the cysteines available for forming intermolecular bonds necessary for producing the glutenin macropolymer. The Dx5 subunit (good-quality related) is notably different from the Dx2 allelic subunit (poor quality) in the presence of an additional cysteine residue ('S' of the disulfide bond in parentheses) at the junction of the N-terminal and repetitive domains. This additional cysteine has been suggested to be related to the quality difference between the alleles, by allowing the formation of longer or more-branched polymers[21].

High-molecular-weight glutenin subunits and breadmaking quality

The elasticity of wheat dough, which depends to a considerable extent on the content, subunit composition and molecular mass of HMW-GSs, is closely related to the breadmaking quality of wheat cultivars[1,2,4,7]. Doughs that have high elasticity and reasonable extensibility are ideal for making bread; doughs that are highly extensible are good for cookies; and doughs with intermediate properties are used for making the flat breads of the Middle East and the Indian subcontinent, or noodles in the Far East. Low gluten elasticity is characteristic of wheat varieties with poor breadmaking qualities. Figure 3 summarizes examples of quantitative correlations of dough physical behavior with the number of active HMW-GS genes. The fact that the HMW-GSs are so important to wheat utilization, and yet are a relatively small percentage of the total seed protein, makes them a good target for genetic engineering. Besides the importance of the absolute amounts of HMW-GS synthesized, it is known that different alleles at the *Glu-1* loci have different levels of effects. For example, the wheat cultivar Chinese Spring is the most important wheat line for genetic studies. However, it is known to be a poor bread wheat. Among its undesirable characteristics are small loaf volume, short peak-mixing time, and rapid breakdown of the mixing curve (caused by loss of elasticity, which is a critical parameter for the baking industry). A set of chromosome substitution lines has been used to substitute chromosomes of the cultivar Cheyenne (a high-quality bread wheat) into Chinese Spring[8]. Tests of dough quality in these Chinese Spring aneuploids showed that the Cheyenne 1D substitution led to larger loaf volumes and less breakdown of the mixing curves. Numerous reports have associated these effects with the HMW-GS allele pair 1Dx5 + 1Dy10, as opposed to the known poor-quality pair 1Dx2 + 1Dy12, which is normally found in Chinese Spring.

The function of high-molecular-weight glutenin subunits

How the HMW-GS polypeptides influence dough quality is not fully understood at the molecular level, but two characteristics of their structure are believed to play important roles. In wheat doughs, the HMW-GSs and LMW-GSs form extensive disulfide-linked polymers. The cysteine residues in the HMW-GSs, necessary for the formation of these glutenin polymers,

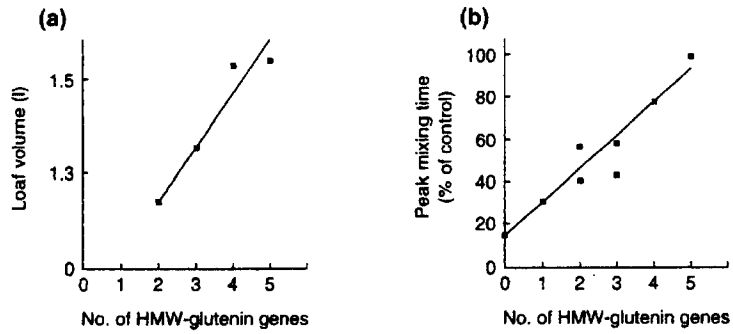


Fig. 3. Effect of high-molecular-weight glutenin subunits (HMW-GSs) on dough processing characteristics. The HMW-GSs are known to have both quantitative and qualitative effects on many of the physical characteristics important for dough formation and good dough quality. Several reports have shown that the number of active HMW-GS genes correlates positively with dough quality. In two reports, near-isogenic lines were produced that varied in the number of active HMW-GS genes and showed that as the normal number of active genes is reduced, important dough characteristics are negatively affected. (a) The loaf volume of bread is a measurement of the ability of the dough to retain gas bubbles during rising and baking. Payne *et al.* found that reducing HMW-GSs decreased loaf volume[32]. (b) As dough is kneaded, its resistance to mixing rises to a peak, but then declines. A general measure of good-quality dough is a longer time to reach this mixing peak. Lawrence *et al.* showed that shorter mixing times result from decreasing the number of HMW-GSs synthesized[33].

are concentrated in the two terminal domains, and the central repetitive domain is composed of a short peptide motif repeated in a tandem series (Fig. 2).

Recently, the application of biochemical and molecular techniques has begun to provide valuable information about high-molecular-weight glutenin function, with careful analysis of disulfide bond patterns[9], and spectrometric and nuclear magnetic resonance studies[10] into their physical structure and the formation of the gluten polymer network. Heterologous expression systems (i.e. not wheat) are also helping to unravel the role of the HMW-GSs in dough functionality. A bacterial system has been used to express a single HMW-GS removed from the normal complex mixture of prolamins in the endosperm[11], and has begun to yield novel information on HMW-GS structure/function relationships[12,13]. This system is also the basis for the construction and expression of a completely synthetic HMW-GS gene analog designed for molecular dissection of glutenin function[14].

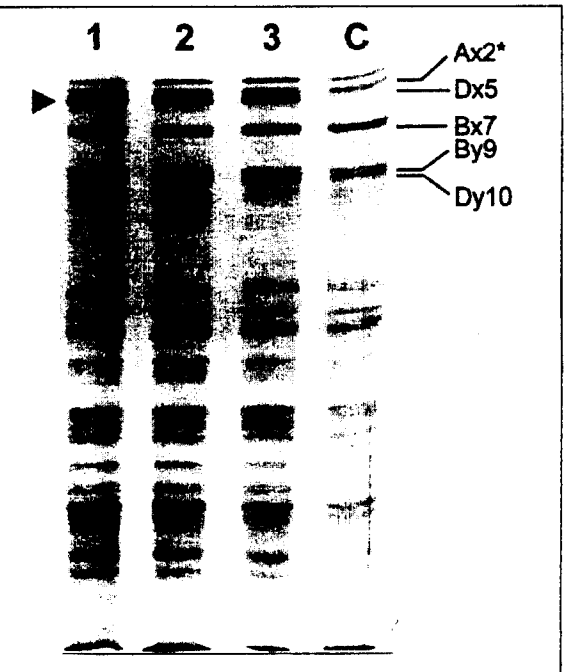


Fig. 4. Expression of a hybrid (Dy10–Dx5) transgenic high-molecular-weight glutenin subunit (HMW-GS) gene (arrowhead) is shown for three independent transgenic wheat lines (gel lanes 1–3). Total protein was extracted, separated using polyacrylamide gel electrophoresis and visualized with the stain Coomassie blue. The control seed-protein pattern (lane C) includes five endogenous HMW-GSs (specific subunit designations are shown on the right). *Adapted from Ref. 25.*

Wheat transformation

The fact that the breadmaking quality of wheat cultivars is determined by specific alleles of HMW-GSs and gene dosage has led to suggestions that the breadmaking quality of wheat cultivars could be improved by the integration and expression of specific HMW-GS genes[15,16]. Until recently, the inability to transform wheat was the main impediment to testing this hypothesis.

There are two important requirements for the production of transgenic plants:

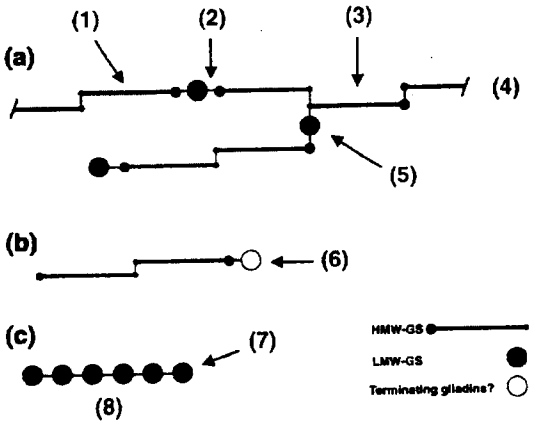
- Stable integration, expression and Mendelian segregation of transgenes.
- Efficient and rapid regeneration of normal, fertile plants from cultured cells and tissues.

During the past decade, both of these requirements have been successfully met for all major cereal species, including wheat[17]. Although several methods have been used for the production of transgenic cereals, high-velocity bombardment of DNA-coated microprojectiles (the biolistics procedure) into cultured immature embryos has consistently provided the best and most credible results[18–23]. Rooted transgenic plants can be obtained in as little as two months, and plants homozygous for the transgene in less than one year[24]. Although the efficiency of transformation is only 1–2%, the technology has developed sufficiently to enable any competent research group experienced in cereal transformation to obtain an adequate number of independent transformants for field evaluation.

Success in the genetic transformation of wheat has been followed by the introduction and expression of HMW-GS genes into wheat. A gene encoding a novel hybrid HMW-GS subunit (Dy10:Dx5), under the control of its native endosperm-specific promoter, has been introduced into the Bobwhite cultivar of wheat[25]. The hybrid HMW-GS accumulated in the endosperm to levels similar to those of native

Box 1 Formation of the glutenin polymer and targets for genetic engineering

The diagram shows three classes of glutenin polymer. The exact structure of these polymers is not established, but it is known that there are three types (a–c). (a) Large polymers formed from a mixture of high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs). These polymers can reach into the tens of millions of daltons. The distribution of linear and branching crosslinks is not known. (b) Shorter polymers containing both HMW-GSs and LMW-GSs. There is actually a continuum of sizes with the largest polymers. The functional differentiation is that the smaller polymers are soluble in salt and in acid–SDS extractions, but the largest polymers are insoluble unless reduced. An increasing proportion of larger polymers consistently correlates with increased dough strength. (c) Polymers of LMW-GSs alone. The reason why polymers of LMW-GSs but not HMW-GSs exist is not known. Two possibilities are a relative excess of LMW-GS synthesis or some specific affinities for disulfide bond formation.



The existing knowledge of the structure and function of the HMW-GSs suggests several targets for genetic engineering. Five of these initial targets are shown (1–5).

(1) Relative amounts of HMW-GS with respect to total seed protein. It is assumed, though not proven, that increasing the numbers of HMW-GS genes will redistribute a constant level of protein synthesis (as against increasing absolute levels of protein produced) in the seed. As more HMW-GSs are synthesized, dough strength tends to increase.

(2) The pattern of disulfide bonds. Altering the number and position of cysteine residues may change the physical characteristics of the gluten matrix.

(3) The repetitive domain. Among the possible modifications are altering repeat domain length and changing the composition and arrangement of the repeat motifs. Thus far there is no convincing evidence for the exact role of this domain. Application of molecular biology and transformation technology should provide significant new insights.

(4) The length of the glutenin macropolymer. Because increasing the proportion of glutenins in the insoluble, or large, polymer fraction correlates with increasing dough strength, strategies to favor such polymers are important for breadmaking. One aspect is the specificity of disulfide crosslinks. Although a detailed description of the crosslinking pattern is not available, there is evidence that the Dx and Dy HMW-GSs have special affinities for one another. If confirmed, this may suggest strategies for constructing genes encoding HMW-GSs that favor polymer extension.

(5) The polymer network. The role of linear as against branched polymers is not known, although it has been suggested that cysteines favoring branching lead to more complex networks and stronger doughs[31].

In addition, newly developed technologies will allow approaches to other important questions in gluten functionality (6–8).

(6) Terminating gliadins. Although the bulk of the smaller polypeptides within the disulfide crosslinked gluten matrix are LMW-GSs, it is known that a small proportion (5–10%) are α- and γ-gliadins[34]. Note that the LMW-GSs are actually members of the gliadin superfamily. The original strict distinction between LMW-GSs and gliadins was established before their evolutionary relationship was established by gene sequencing. The LMW-GSs usually have an odd number of cysteines, but α- and γ-gliadins have an even number. Kasarda[34] has theorized that mutations in cysteine residues could lead to LMW-GSs with only one available cysteine for intermolecular bonds, and gliadins similarly with one available cysteine. In both cases, molecules able to form only a single intermolecular bond would function as polymer chain terminators.

(7) Role of the LMW-GSs in wheat quality. Although the HMW-GSs generally have the highest correlation with quality, the LMW-GSs also play a significant role. Further study is needed on the structure of these subunits similar to that being carried out on the HMW-GSs.

(8) LMW-GS polymers. Although it is known that the polymers are composed entirely of LMW-GSs, neither the organization, size nor specificity of the crosslinks is known. It is important to determine the functional role, if any, of these polymers in gluten functionality.

HMW-GS, and could be distinguished from the latter by its distinct mobility in SDS–polyacrylamide gel electrophoresis (Fig. 4). The hybrid HMW-GS, like the native HMW-GSs, was capable of assembly into high-molecular-weight polymers by forming intermolecular disulfide bonds. The gene encoding HMW-GS 1Ax1 (which is not present in the Bobwhite cultivar of wheat) was also introduced, and the protein accumulated to a high level in the endosperm[26]. Both studies revealed an increase in total HMW-GSs in transgenic seeds, indicating that the accumulation of the transgenic HMW-GS was not at the cost of native HMW-GSs. The high level of expression of HMW-GS genes under the control of their own native promoters in transgenic endosperm was maintained for several generations, with no indication of expression instability.

Engineering novel wheat lines

Having generated a transgenic wheat line, it must be evaluated for three characteristics:

- Does the transgenic locus function correctly?
- How does the transgenic locus affect quality?
- Does the transgenic locus have any effect on agronomic performance?

The ability to introduce novel genes into crop plants by genetic transformation provides new opportunities for cultivar improvement. However, transformation by itself is

insufficient, because the inserted genes must function in a manner appropriate for the desired trait. Transgene instability caused by various factors has been observed in many plant species[27], including the cereals. Another problem is the apparent position effect of the random insertions into the plant genome, resulting in levels of gene expression that vary over several orders of magnitude. These or other causes of variation in gene expression cannot be tolerated in wheat cultivars, because the value of a specific cultivar is dependent on a reproducible expectation of processing characteristics in its flour. A resistance gene might satisfactorily confer a desired phenotype over a wide range of gene activity, but a transgenic HMW-GS gene varying even three-fold because of environmental conditions might not be acceptable. Fortunately, and unexpectedly, the transgenic HMW-GS wheat lines produced so far are showing both very high level and stable synthesis over several generations in controlled environmental conditions[25,26]. Future evaluations must include field trials under varied environmental conditions and the passage of the transgenes through multiple-cross regimes to confirm long-term stability.

Bioengineering wheat for the HMW-GS genes will involve transformation with both existing and modified genes. The resulting loci are expected to be immediately useful in modifying wheat-processing characteristics, although there may be initial uncertainty as to what the effects will be. In addition, the ability directly to modify the pattern of HMW-GS expression will improve our understanding of the organization and function of the gluten matrix. It will then be possible to target and alter specific dough characteristics. Box 1 describes some of the potential targets of HMW-GS genetic engineering.

The uniqueness of the wheat gluten matrix also highlights a problem that is not as crucial to breeding strategies in other cereals. An oft-quoted use of genetic engineering is to increase the amount of essential amino acids in foods, thus increasing their nutritional quality. Because wheat is the most widely grown crop in the world, and can be successfully transformed, it has become an important target for such a strategy. However, dough rheology indicates that such proteins will probably reduce the quality of the resulting wheat lines to such an extent that they may be unusable, because of a loss of dough-forming qualities.

Environmental conditions, such as high temperature, amount of water, pests and pathogens can cause major losses in wheat quality. In some instances, such environmental influences can exceed genotypic variation[28]. Heat shock is particularly associated with decreased quality through a decrease in the degree of glutenin polymerization[29]. Currently, the effects are sporadic in regions that are otherwise suited to wheat cultivation, but global warming could have serious consequences on the range of cultivation of wheat. A better understanding of the molecular basis of dough visco-elasticity would help in developing strategies for mitigating environmental influences on wheat quality.

Molecular approaches, including genetic transformation, provide an opportunity – which may not be possible using conventional methods – for improving wheat processing qualities. The recent success in the introduction and expression of HMW-GS genes into wheat is a promising beginning, but much more work will have to be done in order to understand the role of HMW-GSs before the technology can be used to produce wheat varieties that are superior in their breadmaking characteristics.

References

1 Payne, P.I. (1987) Genetics of wheat storage proteins and the effect of allelic variation on breadmaking quality, *Annu. Rev. Plant Physiol.* 38, 141–153

2 Shewry, P.R. and Miflin, B.J. (1985) Seed storage proteins of economically important cereals, in *Advances in Cereal Science and Technology* (Pomeranz, Y., ed.), pp. 1–83, American Association of Cereal Chemists

3 Wrigley, C.W. (1996) Giant proteins with flour power, *Nature* 381, 738–739

4 Halford, N.G. *et al.* (1992) Analysis of HMW glutenin subunits encoded by chromosome 1A of bread wheat (*Triticum aestivum* L.) indicates quantitative effects on grain quality, *Theor. Appl. Genet.* 83, 373–378

5 Payne, P.I. and Lawrence, G.J. (1983) Catalogues of alleles for the complex gene loci, *Glu-A1*, *Glu-B1*, and *Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheat, *Cereal Res. Commun.* 11, 29–35

6 Anderson, O.D. *et al.* (1988) Structure and analysis of the high-molecular-weight glutenin genes from *Triticum aestivum* L. cv Cheyenne, in *Proceedings of the 7th International Wheat Genetics Symposium* (Miller, T.E. and Koebner, R.M.D., eds), pp. 699–704, Bath Press

7 Shewry, P.R., Halford, N.G. and Tatham, A.S. (1992) High molecular weight subunits of wheat glutenin, *J. Cereal Sci.* 15, 105–120

8 Mansur, L.M. *et al.* (1990) Effects of 'Cheyenne' chromosomes on milling and baking quality in 'Chinese Spring' wheat in relation to glutenin and gliadin storage proteins, *Crop Sci.* 30, 593–602

9 Köhler, P., Belitz, H.D. and Wieser, H. (1993) Disulfide bonds in wheat gluten: further cystine peptides from high molecular weight (HMW) and low molecular weight (LMW) subunits of glutenin and from γ-gliadins, *Z. Lebensm.-Unters. Forsch.* 196, 239–247

10 Belton, P.S. *et al.* (1994) ^{1}H and ^{2}H NMR relaxation studies of a high M_r subunit of wheat glutenin and comparison with elastin, *J. Cereal Sci.* 19, 115–121

11 Galili, G. (1989) Heterologous expression of a wheat high molecular weight glutenin gene in *Escherichia coli*, *Proc. Natl. Acad. Sci. U. S. A.* 86, 7756–7760

12 Bekes, F. *et al.* (1994) The contributions to mixing properties of 1D HMW glutenin expressed in a bacterial system, in *Improvement of Cereal Quality by Genetic Engineering* (Henry, R. and Ronalds, J.A., eds), pp. 97–103, Plenum Press

13 Shani, N. *et al.* (1992) Role of the amino- and carboxy-terminal regions in the folding and oligomerization of wheat high molecular weight glutenin subunits, *Plant Physiol.* 98, 433–441

14 Anderson, O.D., Kuhl, J.C. and Tam, A. (1996) Construction and expression of a synthetic wheat storage protein gene, *Gene* 174, 51–58

15 Flavell. R.B. *et al.* (1989) Genetic variation in wheat HMW glutenin subunits and the molecular basis of breadmaking quality, *Biotechnology* 7, 1281–1285

16 Shewry, P.R. *et al.* (1995) Biotechnology of breadmaking: unraveling and manipulating the multi-protein gluten complex, *Biotechnology* 13, 1185–1190

17 Vasil, I.K. (1994) Molecular improvement of cereals, *Plant Mol. Biol.* 25, 925–937

18 Vasil, V. *et al.* (1992) Herbicide resistant fertile transgenic wheat plants obtained by micro-projectile bombardment of regenerable embryogenic callus, *Biotechnology* 10, 667–674

19 Vasil, V. *et al.* (1993) Rapid production of transgenic wheat plants by direct bombardment of cultured immature embryos, *Biotechnology* 11, 1553–1558

20 Weeks, J.T., Anderson, O.D. and Blechl, A.E. (1993) Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum*), *Plant Physiol.* 102, 1077–1084

21 Becker, D., Brettschneider, R. and Lörz, H. (1994) Fertile transgenic wheat from microprojectile bombardment of scutellar tissue, *Plant J.* 5, 299–307
22 Nehra, N.S. *et al.* (1994) Self-fertile transgenic wheat plants regenerated from isolated scutellar tissues following microprojectile bombardment with two distinct gene constructs, *Plant J.* 5, 285–297
23 Srivastava, V., Vasil, V. and Vasil, I.K. (1996) Molecular characterization of the fate of transgenes in transformed wheat (*Triticum aestivum* L.), *Theor. Appl. Genet.* 92, 1031–1037
24 Altpeter, F. *et al.* (1996) Accelerated production of transgenic wheat (*Triticum aestivum* L.) plants, *Plant Cell Rep.* 16, 12–17
25 Blechl, A.E. and Anderson, O.D. (1996) Expression of a novel high molecular weight glutenin subunit gene in transgenic wheat, *Nat. Biotechnol.* 14, 875–879
26 Altpeter, F. *et al.* (1996) Integration and expression of the high-molecular-weight glutenin subunit *1Ax1* gene into wheat, *Nat. Biotechnol.* 14, 1155–1159
27 Meyer, P. and Saedler, H. (1996) Homology-dependent gene silencing in plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47, 23–48
28 Peterson, C.J. *et al.* (1992) Genotype and environmental effects on quality characteristics of hard red winter wheat, *Crop Sci.* 32, 98–103
29 Ciaffi, M. *et al.* (1996) Effect of heat shock during grain filling on the gluten protein composition of bread wheat, *J. Cereal Sci.* 24, 91–100
30 Lookhart, G.L., Hagman, K. and Kasarda, D.D. (1993) High-molecular-weight glutenin subunits of the most commonly grown wheat cultivars in the U.S. in 1984, *Plant Breed.* 110, 48–62
31 Greene, F.C. *et al.* (1989) Analysis of possible quality-related sequence variations in the D glutenin high molecular weight subunit genes of wheat, in *Proceedings of the 7th International Wheat Genetics Symposium* (Miller, T.E. and Koebner, R.M.D., eds), pp. 735–740, Bath Press
32 Payne, P.I. *et al.* (1987) The use of near-isogenic lines with different HMW glutenin subunits in studying bread-making quality and glutenin structure, in *3rd International Gluten Workshop*, pp. 216–226
33 Lawrence, G.J., Macritchie, F. and Wrigley, C.W. (1988) Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, *J. Cereal Sci.* 7, 109–112
34 Kasarda, D.D. (1989) Glutenin structure in relation to wheat quality, in *Wheat is Unique* (Pomeranz, Y., ed.), pp. 277–302, American Association of Cereal Chemists

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Initiation of microspore embryogenesis by stress

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Microspores have the remarkable capacity to develop into haploid plants via embryogenesis *in vitro*. Stress treatment acts as a trigger for inducing this sporophytic pathway, preventing the development of fertile pollen (gametophytic pathway). The doubled haploids generated are completely homozygous, and represent an important tool for research in plant genetics and breeding. In addition, microspore embryogenesis can be used to study plant embryogenesis and phase transitions during the alternation of generations in plants. Microspore culture also allows stress to be analyzed in the novel context of cell cycle regulation and plant development.

Pollen development follows a tightly controlled sequence of events that can be divided into two major processes: microsporogenesis and microgametogenesis. Microsporogenesis begins with meiosis and ends with the formation of polarized haploid microspores. During microgametogenesis, the unicellular microspore divides asymmetrically, resulting in a young pollen grain containing a vegetative cell and a generative cell, which differentiates into a mature, bicellular or tricellular pollen grain[1].

One of the original techniques used to study pollen development has been anther culture *in vitro*[2]. Surprisingly, no mature pollen developed in microspore-containing, *in vitro*-cultured anthers of *Datura innoxia*, but embryos with the typical appearance of dicotyledons emerged from the locules[3]. These embryos were derived from the microspores and developed into plants with the gametic (haploid) set of chromosomes. This developmental pathway is referred to as microspore embryogenesis, irrespective of whether the embryos originate from unicellular microspores or immature bicellular pollen grains. Since this original discovery, interest has developed in using anther culture to produce homozygous doubled haploid plants for genetic and developmental studies, as well as for plant breeding[2,4]. At present, the list of species in which microspore-derived embryos and/or doubled haploids have been produced includes species from many families, including most major crop plants, although the legumes are a notable exception[4].

Anther culture is technically simple, and is still used in many commercial labs for doubled haploid production, but in recent years the use of isolated microspore culture has emerged as an alternative. Microspore cultures offer the advantage that the sporophytic anther wall tissues do not interfere in the process, and the development of the embryo can thus be followed directly. This breakthrough has been

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