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REVIEW ARTICLE

Molecular characterization and functional analysis of elite genes in wheat and its related species

JIRUI WANG[1], PENGFEI QI[1], YUMING WEI[1], DENGCAI LIU[1], GEORGE FEDAK[2] and YOULIANG ZHENG[1]*

[1]*Triticeae Research Institute, Sichuan Agricultural University, Yaan 625014, Sichuan, People's Republic of China*
[2]*Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario, K1A 0C6, Canada*

Abstract

The tribe *Triticeae* includes major cereal crops (bread wheat, durum wheat, triticale, barley and rye), as well as abundant forage and lawn grasses. Wheat and its wild related species possess numerous favourable genes for yield improvement, grain quality enhancement, biotic and abiotic stress resistance, and constitute a giant gene pool for wheat improvement. In recent years, significant progress on molecular characterization and functional analysis of elite genes in wheat and its related species have been achieved. In this paper, we review the cloned functional genes correlated with grain quality, biotic and abiotic stress resistance, photosystem and nutrition utilization in wheat and its related species.

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Introduction

Wheat and its related species, including more than 250 perennial and 75 annual species forming the tribe *Triticeae* in the grass family (*Poaceae*) have proved themselves to be one of the most indispensable plant groups for human welfare. There are five major cereal crops, namely bread wheat, durum wheat, triticale (*Triticosecale*, an artificially synthesized species), barley and rye, all of which have been utilized for staple food, beverages and forage in a multitude of ways in the history of mankind. Due to their great agronomic importance, the exploitation of cloned elite genes in *Triticeae* is one of the most feasible approaches for wheat improvement.

Wheat and its wild relatives carry genes for yield improvement, grain quality enhancement, biotic and abiotic stress resistance, which could be applied as a useful gene pool for wheat improvement. Considerable variability was found for resistance to insects, rust (leaf, stem and stripe rusts), powdery mildew and *Fusarium* head blight (FHB). In recent years, significant progress on molecular characterization and functional analysis of elite genes has been achieved with different molecular approaches in wheat and its related species (table 1). In this review, we summarize the advances in research work on the cloned genes of grain quality, biotic and abiotic stress resistance, photosystem and nutrition utilization, with emphasis on genes for grain quality.

*For correspondence. E-mail: ylzheng@sicau.edu.cn.

Genes for grain quality

Improvement of grain quality is a major breeding objective for common wheat breeding. In particular, the structural composition of the mature endosperm, including protein content, grain hardness and starch quality, are important factors in determining end-use quality of wheat flour. Because of the wide range of applications, the processing quality of wheat has been the subject of immense volume of research and was one of the first targets for genetic modification (GM) in the late 1990s. So far these studies have focused on two aspects of quality that are determined principally by the grain protein composition: dough strength and grain texture (hardness) (Shewry and Jones 2005).

Baking quality

Of all cereal crops, wheat is important in the human diet, and a wide variety of foods have been developed to take advantage of the nutritional properties of wheat flour.

Keywords. functional genes; grain quality; biotic stress; abiotic stress; Triticeae.

Table 1. Molecular cloning approaches employed in wheat functional gene characterization.

Methods	Functional genes	Reference
Homology-based cloning	*TaLon1, TaZF, Glu-Ee1.5*	Liu *et al*. (2005), Wan *et al*. (2004), Wang *et al*. (2004)
Map-based cloning	*Lr21, PM3, Gpc-B1*	Huang *et al*. (2003), Yahiaoui *et al*. (2004), Uauy *et al*. (2006)
Suppression subtractive hybridization (SSH)	*Wali1-5, TaRab*	Snowden and Gardner (1993), Yao *et al*. (2005)
Differential display of mRNA reverse transcription PCR (DDRT-PCR)	*Hsp16.9*, Rhd3	Joshi and Nguyen (1996), Shan *et al*. (2005)
Electric PCR cloning (ePCR)	*WDAI*	Wang *et al*. (2005)
In situ hybridization (ISH)	*Histone-H4*	Drea *et al*. (2005)

The unique properties of wheat flour primarily depend on seed storage proteins, the great majority of which comprises of glutens (also named prolamins) with good solubility in 70%–90% ethanol. Glutens can be divided into glutenins (HMW-GSs and LMW-GSs) and gliadins (α-(α/β), γ-gliadins and ω-gliadins).

HMW-GSs are significant in determination the wheat grain processing quality due to their compositional effects on the elasticity of wheat dough. Shewry *et al*. (2002) reported that two features (the number and distribution of disulphide bonds and the properties and interactions of the repetitive domains) of HMW-GSs are the major determinants of wheat baking quality, and might be relevant to their participation as gluten elastomers in the baking quality of dough. HMW-GSs coding genes have been isolated from many Triticeae species, such as *Aegilops, Crithopsis, Dasypyrum, Hordeum, Secale, Taeniatherum, Thinopyrum*, and diploid and tetraploid wheat (Wan *et al*. 2002; Yan *et al*. 2002; De Bustos and Jouve 2003; Liu *et al*. 2003; Sun *et al*. 2004; Wang *et al*. 2006; Cao *et al*. 2007; Liu *et al*. 2007). Further, some of the subunits from wheat related species might have a potential good quality profile, which could be used to enhance the baking quality of common wheat through transgenic approaches (see review, Wang *et al*. 2007b). LMW-GSs on the other hand play an important role in the formation of gluten polymer. Masci *et al*. (1998) suggested that the long repetitive regions in LMW-GS contribute to the baking quality. Gliadins are monomeric proteins of 30–78 kDa with poor solubility in dilute salt solutions, but good solubility in alcohol. Negative, positive and/or no correlation has been reported in functional tests. Khatkar *et al*. (2002a,b) suggested that the interactive behaviour of gliadins with gluten proteins through covalent and noncovalent binding might be important in determining bread-making quality. It seems likely that α-gliadins, β-gliadins and γ-gliadins have a greater tendency to interact with gluten than ω-gliadins because of the presence of cysteines in these types of gliadins. Since hundreds of storage proteins have been characterized from wheat and its related species (see table 1, A–D of appendix), the knowledge of the contribution of each type of storage protein should be obtained by transgenic research and/or the addition of the corresponding protein classes to flour, with the intention of altering the relative proportions of different components in flour and studying their effects.

To enhance the quality of common wheat, the expression of additional storage protein gene copies in transgenic wheat has been carried out. To determine the relationship between dough quality and amounts and properties of the LMW subunits, pasta wheat was transformed with three genes encoding proteins, which differ in their numbers or positions of cysteine residues. It was demonstrated that the ability to transfer specific LMW glutenin subunits could greatly assist in the elucidation of their individual contributions to the functionality of the complex gluten system (Tosi *et al*. 2004). Recently, Tosi *et al*. (2009) determined the precise routes of trafficking and deposition of individual gluten proteins in developing wheat grain, with proteins either being transported via Golgi apparatus into the vacuole or accumulating directly within the lumen of the ER (Tosi *et al*. 2009).

Commercial wheat was successfully engineered to biosynthesize the HMW glutenin subunit gene *Glu-Ax1*, and which resulted in an increase in the total amount of HMW subunit protein in the seeds of transgenic wheat plants (Yao *et al*. 2006). There are both quantitative and qualitative effects of HMW subunits on the quality of the grain. Barro *et al*. (1997) transformed bread wheat in order to increase the proportions of the HMW subunits and improve the functional properties of the flour. The T2 seeds expressing transgenes for one or two additional HMW subunits showed stepwise increases in dough elasticity, demonstrating the improvement of the functional properties of wheat by genetic engineering (Barro *et al*. 1997). When transgenic lines have been evaluated under field conditions, it is found that differences between transgenic lines and their parents are small, and could be eliminated by backcrossing transgenic lines with their parents and selecting for the wanted genotype (Barro *et al*. 2002). HMW glutenin subunit genes, *Glu-Ax1* and *Glu-Dx5*, were also successfully introduced and either expressed or overexpressed into a commercial wheat cultivar that already expresses five subunits (Alvarez *et al*. 2000). In our lab, a y-type HMW-GS *Glu-Ee1.5* obtained from *Elytrigia elongata* was transformed in to Chinese wheat, and we are presently

studying the quality variation between the transgenic wheat and original one (Q. T. Jiang, J. R. Wang, Y. M. Wei and Y. L. Zheng, unpublished data).

Grain texture

Endosperm texture (hardness or softness of a grain) is also one of the primary determinants of wheat's end-product quality. Based on grain hardness, wheat can be simply classified into either soft or hard. Hard wheat is better suited for yeast-leavened bread baking, while soft wheat is preferred for cookies, cakes and pastries.

Grain hardness is simply inherited and primarily controlled by the hardness (*Ha*) gene, which is located on the short arm of chromosome 5D of common wheat (Chen *et al*. 2007). Friabilin, a starch granule protein associated with wheat endosperm hardness/softness, was defined as a 15000-kDa protein. It is comprised of a mixture of components including two major tryptophan-rich proteins called puroindoline a (*Pina*) and b (*Pinb*) (Bettge *et al*. 1995), which are encoded by the genes located at the *Ha* locus. Near the *Ha* locus, there are extra genes encoding grain soft protein (*GSP*). Hundreds of *Pina, Pinb* and *GSP* genes have been isolated from many species, such as *T. aestivum, T. turgidum, T. urartu, T. monococcum, T. timopheevii, T. zhukovskyi, Ae. tauschii, Ae. speltoides, Secale* and *Hordeum* (Morris 2002). Previous work has demonstrated that changing grain hardness by overexpressing puroindolines in transgenic isolines (Hogg *et al*. 2005; Martin *et al*. 2007, 2008) and allelic variation at *Pin* loci in a recombinant inbred population (Martin *et al*. 2001) affected bread quality. In wheat, transgenic expression of wild-type *Pinb-D1a* sequence in the hard spring wheat 'Hi-Line' complemented the glycine-to-serine mutation (*Pinb-D1b* allele) resulting in a soft phenotype (Beecher *et al*. 2002). Hogg *et al*. (2004) expressed *Pina-D1a, Pinb-D1a*, or both in the same Hi-Line background. Expression of either *Pinb*, or both *Pina* and *Pinb* gave a soft phenotype, while *Pina* alone was intermediate in grain texture. Transgenic expression of wild type *Pina-D1a* sequence in the hard wheat 'Bobwhite' which has the *Pina-D1b* (null) allele also gave a soft phenotype (Martin *et al*. 2006). The highly efficient expression vector *pUBPa* harbouring puroindoline a (*Pina*) was introduced into the bread wheat via biolistic transformation (Xia *et al*. 2008). SDS-PAGE analysis showed that the PINA protein was absent in three transgenic lines, indicating that the endogenous *Pina* gene most likely had been co-suppressed by the overexpression of the *Pina* transgene.

Starch biosynthesis

Seed starch is the major storage compound in cereals providing as much as 80% of the calories consumed by mankind. This starch is also a major source of feed, fiber, biofuels, and biopolymers in many industrial applications. The molecular basis of starch synthesis should be understood as a prerequisite for improving and modifying starch properties. Starch is composed of two glucose polymers (amylose and amylopectin), which share the same basic glucan structure but differ in length and degree of branching. The synthesis of starch in the endosperm of the higher plants occurs within the amyloplast and involves the action of at least four types of enzyme activities, ADP-glucose pyrophosphorylase (AGP), starch synthases, branching enzymes and debranching enzymes (Morell *et al*. 2001). The cloning and characterization of the key genes controlling the starch biosynthesis pathway in wheat have now been reported.

The enzyme that controls starch biosynthetic pathway was characterized (Hannah 1997; Preiss 1997). ADP-glucose pyrophosphorylase is the product of the Agp-L and Agp-S subunits, and catalyses the rate-limiting reaction in starch biosynthesis in plants (Preiss and Romeo 1994). The large and small subunits of endosperm AGP of maize are encoded by Shrunken2 (Sh2) and Brittle2 (Bt2) respectively (Bae *et al*. 1990; Bhave *et al*. 1990). Inhibition of AGP activity appears to limit starch biosynthesis and yield in crop plants. Because AGP is the rate-limiting step in starch biosynthesis, an increase in AGP activity within the endosperm of wheat seeds should increase developing seed sink strength and overall plant productivity (Smidansky *et al*. 2002). Wheat was transformed with modified maize *Sh2* gene (Sh2r6hs), which encoded an altered AGP large subunit, giving rise to an AGP heterotetramer with decreased sensitivity to inhibition and more stable subunit interactions (Smidansky *et al*. 2002). *Sh2r6hs* transgenics were field tested over four growing years, in three locations, with varying planting density and irrigation and the results indicated significant yield increases in space-planted, irrigated environments than densely planted, rained environments, suggesting that limited abiotic resources may subsequently limit *Sh2r6hs*-associated yield enhancement (Meyer *et al*. 2007). Further, modifications in Agp-L can lead to measurable changes in starch biosynthesis and grain yield (Smidansky *et al*. 2003).

Sucrose transporter (SUT) controls sucrose uptake and downloading into sink cells (Lemoine 2000). The production of the starch components, amylose and amylopectin, is controlled by granule-bound starch synthase (Waxy) and soluble starch synthases (SS), respectively (Shure *et al*. 1983). Starch synthases catalyse the transfer of the glucose moiety from ADP-glucose to the reducing end of a pre-existing α-1,4-linked glucan polymer. SS were firstly isolated from hexaploid wheat and *Ae. tauschii* by Li *et al*. (1999). The entire sequence of the SS-I encodes a polypeptide of 647 amino-acid residues that shows 81% identity to the amino-acid sequences of SSI-type starch synthases from rice. The coding genes of Waxy from three genomes were also isolated and characterized, which showed high identity between each other (Murai *et al*. 1999).

Recently, genome-specific primers for wheat *AGP, SUT, Wx, Ss* genes were developed, which could help us to distinguish the genes from A, B, and D genomes respectively (Blake *et al*. 2004). The transcriptional control of storage

starch synthesis in developing wheat caryopsis was analysed (Stamova *et al*. 2009). A biphasic pattern was observed in rates of starch and protein accumulation which paralleled changes in global gene expression. Metabolic and regulatory genes that show a pattern of expression similar to starch accumulation and granule size distribution were identified, suggesting their coinvolvement in these biological processes (Stamova *et al*. 2009). A global gene expression profiling experiment with the analysis of soluble sugar accumulation, starch content, and starch granule particle size distribution were carried out, which gave us the overview of the expression level of genes coding for key enzymes involved in starch biosynthesis.

Seed nutritional content

The sucrose transporter gene *HvSUT* of barley, which functions in sucrose uptake into seeds during grain filling, was cloned and expressed (Weschke *et al*. 2001; Sivitz *et al*. 2005). It was shown that the *HvSUT1* was more selective for α-glucosides over β-glucosides than the *Arabidopsis* sucrose transporter *AtSUC2*. Wheat lines that contain the barley *HvSUT1* gene might lead to greater numbers of carbon structures and consequently to increased protein synthesis. The rapid induction of *HvSUT1* gene expression in caryopses at approximately 5–6 days after fertilization coincides with increasing levels of sucrose as well as sucrose synthase mRNA and activity, and occurs immediately before the onset of rapid starch accumulation within the endosperm (Weschke *et al*. 2001). Starch biosynthesis requires sucrose to be imported into the endosperm as a direct precursor for starch synthesis, and to promote storage-associated processes.

The reintroduction of the functional gene(s) from the wild species into commercial wheat varieties has the potential to increase the nutritional value of a large proportion of currently cultivated wheat varieties. Genes have been identified from wild wheat that could increase protein and micronutrient content of its cultivated cousin by 10%–15%, and could soon be used in food products with enhanced nutritional value (Uauy *et al*. 2006). The *NAM-B1* gene (*Gpc-B1*) is a NAC transcription factor that affects grain nutrient concentrations in wheat as it influence an increased efflux of nutrients from the vegetative tissues and also a higher partitioning of nutrients to grain (Uauy *et al*. 2006; Waters *et al*. 2009). The *Gpc-B1* allele was originally introgressed into hexaploid wheat from *T. dicoccoides* (Mesfin *et al*. 1999), and is associated with increased levels of grain protein, zinc, and iron as a consequence of accelerated senescence and increased nutrient mobilization from leaves to the developing grains. In addition, it has been characterized and cloned from wild emmer wheat (Uauy *et al*. 2006). It could accelerate grain maturity and increase protein and micronutrient content. Subsequently, it will help us to deal with the human problem of zinc and iron deficiency and provide an adequate protein supply to people. However, this gene was found to be nonfunctional in all the cultivated pasta and bread wheat varieties, which suggests that the functionality of the gene was lost during the domestication of wheat. An RNAi line with reduced expression of *NAM* genes has lower grain protein, iron (Fe), and zinc (Zn) concentrations. To determine whether decreased remobilization, lower plant uptake, or decreased partitioning to grain are responsible for this phenotype, mineral dynamics were quantified in wheat tissues throughout grain development. The results suggest that a major effect of the *NAM* genes is an increased efflux of nutrients from the vegetative tissues and a higher partitioning of nutrients to grain (Waters *et al*. 2009).

Genes for abiotic stress

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity, and oxidative stress are significant causes of crop loss worldwide and are serious threats to agriculture. Abiotic stresses are often interconnected, and could induce similar cellular damage. To maintain growth and productivity, plants must adapt to stressful conditions and exercise specific tolerance mechanisms. Plant modification for enhanced tolerance is mostly based on the manipulation of genes that protect and maintain the function and structure of cellular components. Present engineering strategies rely on the transfer of one or several genes that are either involved in signalling and regulatory pathways, or that encode enzymes present in pathways leading to the synthesis of functional and structural protectants, or that encode stress-tolerance-conferring proteins (Wang *et al*. 2003).

Salinity

The growth of the plant is inhibited by salt, which reduces the ability to take up water. Salt can also enter the transpiration stream and eventually injure cells in the transpiring leaves, further reducing growth. This is the salt-specific or ion-excess effect of salinity (Munns 2005). Wheat is grown under irrigated and rain-fed conditions which are threatened by salinization. The wheat gene pool generally has low tolerance to salinity and this restricts advancement in developing salt tolerant germplasm (Salam *et al*. 1999). However, some alien species, such as *Thinopyrum, T. turgidum, H. marinum, Secale cereale, Leymus chinensis, H. brevisubulatum*, and barley are more salt-tolerant than wheat (Liu *et al*. 2001; Garthwaite *et al*. 2003; Colmer *et al*. 2005; Wang X. Q. *et al*. 2007). *Thinopyrum* has received the most attention as a source for improving salt tolerance in wheat (Colmer *et al*. 2006). In this context, genes for salinity-tolerance from *Th. elongatum* have already been transferred into wheat (Chen *et al*. 2000).

Some candidate genes from wheat-related species could increase the salt tolerance of common wheat. Those genes control salt uptake and transport, making a plant grow more quickly in saline soil. These genes are thought to have an osmotic or protective function. A vacuolar Na^+/H^+ antiporter gene, *AeNHX1*, was isolated from *Th. elongatum* and transformed into *Arabidopsis* and *Festuca* plants. The overexpres-

sion of the *AeNHX1* gene could promote salt tolerance of *Arabidopsis* and *Festuca*, thus expression of this gene was important in salt tolerance (Qiao *et al*. 2007). Transgenic wheat expressed a vacuolar Na^+/H^+ antiporter gene *AtNHX1* from *Arabidopsis thaliana* (Xue *et al*. 2004). Field trial revealed higher grain yields and heavier and larger grains in the field of saline soils with the electrical conductivity values of soil saturation extracts (EC_e) of 10.6 and 13.7 dS m^{-1}. Further, the transgenic lines accumulated a lower level of Na^+ and a higher level of K^+ in the leaves than non-transgenic plants under saline conditions (100 and 150 mM NaCl). Hence these results indicate the feasibility of increasing salt tolerance of wheat and grain yield by enhancing the level of the vacuolar Na^+/H^+ antiporter.

A sodium transporter (*HKT7*) is a candidate salt-tolerance gene for *Nax1* that could reduce Na^+ concentration in leaf blades by retaining Na^+ in the sheaths in *durum* wheat. *TmHKT7-A2* from *durum* wheat is reported to control Na^+ unloading from xylem in roots and sheaths (Huang *et al*. 2006). *HVA1*, a barley group III (D-7 family) LEA protein, is highly induced by ABA-stress in barley aleurone layers and related to salt tolerance (Hong *et al*. 1992). This barley late embryogenesis abundant protein gene *HVA1* was successfully transformed into *T. aestivum, T. durum* and mulberry to enhance the ability of tolerance to salinity and water stress (Patnaik and Khurana 2003).

Drought

Drought is the shortage of water availability, and is based on precipitation and soil-moisture storage capacity in quantity and distribution during the life cycle of wheat (Mitra 2001). Bread wheat has low drought resistance, although wild relatives of wheat have an excellent ability to resist drought. Genes for drought tolerance have been discovered in a number of wild wheat relatives and their functions have been investigated. Species in the genus *Thinopyrum* showed good drought tolerance (Dong 1981; Zhang *et al*. 2007). The salinity related gene *HVA1* can also improve the drought tolerance of barley (Li *et al*. 2006). Sivamani *et al*. (2000a) introduced *HVA1* into spring wheat cultivar Hi-Line using biolistics. The transgenic plants under controled environmental conditions, had higher water use efficiency values, significantly greater total dry mass, root fresh and dry weights, and shoot dry weight compared to the controls under soil water deficit conditions. Four selected transgenic lines were tested further, in nine field experiments over six cropping seasons and one promising line was selected for commercialization also (Bahieldin *et al*. 2005). Moreover, the DHN-5 protein in *durum* wheat is also related to drought tolerance (Brini *et al*. 2007). In the resistant wheat variety, a series of highly phosphorylated DHN-5 were detected whereas in the sensitive variety that were weakly detectable. Accumulation of phosphorylated DHN-5 mainly in the resistant wheat variety suggested a role of it in preservation of cell integrity during late embryogenesis and desiccation.

Cold

Many plants, including Triticeae species, exhibit an increase in freezing tolerance in response to low, non-freezing temperatures, through a phenomenon known as cold acclimation. The physiological, biochemical, and molecular processes involved in the attainment of cold acclimation have been studied extensively, but a complete understanding of the functions of the various genes induced by low temperature is still lacking (Thomashow 1999).

A number of genes are believed to respond to cold and freezing temperatures during cold acclimation including *COR* (cold-regulated) genes (Ganeshan *et al*. 2008). It has been suggested that the C-repeat binding factor (CBF) / dehydration-responsive element binding protein 1 (DREB1) regulon is the most important transcriptional unit involved in cold acclimation in plants (Nakashima and Yamaguchi-Shinozaki 2006). In barley, 20 CBF genes were identified, 11 of which were assigned to two tandem clusters on chromosome arm 5HL (Skinner *et al*. 2006). In diploid wheat, 11 CBF genes form a cluster within a 0.8 cM region located on chromosome $5A^m$ (Miller *et al*. 2006).

Screening of a wheat cold-acclimated cDNA library resulted in the identification of a novel cold-regulated gene called *Wcs19* (Chauvin *et al*. 1993). In an effort to gain a better insight into the structural and functional features of this gene family, *Wcs19* homologs and orthologs in barley, rye, and wheat were also characterized. The molecular and biochemical analyses revealed that this gene family encodes chloroplastic proteins related to group 3 LEA proteins (NDong *et al*. 2002). Moreover, expression of the low-temperature induced *Wcs120* gene of wheat has been associated with freezing tolerance and was used to study mRNa and protein accumulation in wheat-rye and wheat-crested wheatgrass (*Agropyron cristatum*) interspecific combinations during cold acclimation (NDong *et al*. 2002).

Khanna and Daggard (2006) produced transgenic wheat lines via biolistics using immature embryos of spring wheat cultivar Seri 82 with a recombinant antifreeze gene, *rAFPI*, targeted to the apoplast using a Murine leader peptide sequence from the mAb24 light chain for retention in the endoplasmic reticulum using a C-terminus KDEL sequence. The transgenic wheat line T-8, with apoplast-targeted antifreeze protein exhibited highest levels of antifreeze activity and also provided significant freezing protection even at temperatures as low as $-7^\circ C$.

Genes for biotic stress resistance

Stresses from biotic factors, especially fungi, viral and insects, are also very important factors that reduce wheat productivity.

Fungal disease

A large number of fungal diseases are known to cause considerable damage to wheat production worldwide. Resistance

breeding for major pathogen diseases, such as rusts, powdery mildew and *Fusarium* head blight (FHB), has been the most important challenges for wheat breeders. These efforts have led to the identification of resistance genes from wheat and its related species to virulent mutating pathogens (Friesen *et al*. 2006). Hundreds of related resistance genes have been molecularly mapped and many genes in the plant ET, SA and JA pathways correlating with fungal resistance were obtained, while only a few functional genes directly resisting the fungi were discovered.

Map-based cloning and functional genetic studies in model plant systems have become easier with the availability of whole-genome sequences and provide fundamental knowledge for understanding plant growth and environmental response. But in crops with large polyploid genomes, cloning the functional genes and deploying them for crop improvement presents special challenges. In wheat especially, an average ratio of 4.4 Mb/cM of physical/genetic distance presents an almost impossible task for map-based cloning of genes in wheat. Fortunately, in recent years, some genes associated with fungal resistance were map-based cloned from wheat and its related species (table 2). Fifteen such genes have been cloned to date (table 2). These include genes for leaf rust resistance (five genes), powdery mildew resistance (seven genes), stem (one gene) and stripe rust resistance (two genes). Moreover, disease-resistant genes from Triticeae, such as the stilbene synthase gene *vst1* obtained from barley have been introduced into wheat (Leckband and Lorz 1998). Transgenic wheat plants stably expressing another antifungal barley seed class II chitinase gene (*PR3*) showed increased resistance to powdery mildew (Bliffeld *et al*. 1999), but even more significant protection was obtained with the introduction of the gene for an apoplastic ribosome-inactivation protein (RIP) from barley (Yahiaoui *et al*. 2006). The expression of genes for an anti-fungal protein from *Aspergilus giganteum* and a barley class II chitinase were shown to significantly reduce the formation of powdery mildew and leaf rust in wheat (Oldach *et al*. 2001).

Viral resistance

Wheat streak mosaic (WSM) is a serious disease affecting wheat in wheat producing countries. This disease, which causes serious loss of wheat yield, is caused by wheat streak mosaic virus (WSMV) of the family Potyviridae. It was suggested that expression of viral gene sequences in transgenic plants might provide a means of disrupting the viral life cycle, while leaving host functions unaffected. Thus wheat immature embryos were transformed with the replicase gene (*NIb*) and coat protein gene (*CP*) of WSMV through biolistic method (Sivamani *et al*. 2000b, 2002). Transgenic plants showed various degree of resistance to WSMV, and some had milder symptoms and lower virus titer than control plants after inoculation.

Genes for insect resistance

Wheat productivity is threatened by insects, including aphids, Hessian fly, locusts, beetles, moths, etc. (Curtis *et al*. 2002). However, research on the introduction of insect resistance genes into wheat has so far been very limited. Increased insect mortality is caused by lowering protease activity in insect guts, resulting in reduced levels of available amino acids. In cereal seeds, α-amylase inhibitors are known to be organized as one major family on the basis of the homology of their amino acid sequences. The monomeric and dimeric α-amylase inhibitor genes (*WMAI* and *WDAI*) have been well characterized from wheat, barley, rye and *Aegilops*, and could be used through plant genetic engineering for weevil control (Wang *et al*. 2007a, 2008). The barley trypsin inhibitor gene *CMe* (*BTAI-CMe*) has been introduced into the wheat (Altpeter *et al*. 1999). Expression and functional integrity of *BTAI-CMe* in transgenic seeds were demonstrated, along with a significant reduction in the survival of the *Sitotroga ceralella*, a major pest of stored wheat grain reared on transgenic seeds expressing *BTAI-CMe*. The recent characterization and expression study of a novel wheat gene (*Hfr-3*) encoding a putative chitin-binding lectin that is associated with resistance to Hessian fly, a major pest that causes considerable damage is another example of a specific insect resistance gene (Giovanini *et al*. 2007).

Other important genes

Photosystem

Improving photosynthesis is a target trait for maximizing wheat productivity. Characterization of key enzymes in the Calvin cycle is a very important objective. Rubisco, one of the key enzyme involved in photosynthesis, consists of two subunits, and catalyses carboxylation. Rubisco is encoded by *rbcL* and *rbcS* genes, which have been isolated in many wild relatives, such as *Hordeum, Secalee, Aegilops, Arena*, and *Thinopyrum* (Guo *et al*. 1997; Hao *et al*. 2007). The efficiency of photosynthesis might be increased by these genes by altering the carboxylase/oxygenase ratio and thus maximizing the carboxylase activity.

Rubisco activase (RCA) mediates assembly of the two subunits and regulates rubisco's activity. In barley, RCA has α and β subunits and their encoding genes have been characterized (Sabine and Raymond 1991). *RcaA* produces two mRNAs, which encode 42 kDa and 46 kDa subunits; *RcaB* encodes a 42-kDa β subunit. The transforming *RcaA* gene could potentially increase the photosynthetic activity of wheat and thus its productivity. The final stage of the Calvin cycle is to regenerate RuBP. In that cycle, triosephosphate isomerase (TPI) is the key enzyme, and it is also important for the biosynthesis of starch .The photosynthetic efficiency of transgenic *Anabaena* sp with *tpi* is higher than that of the wild type (Kang *et al*. 2004). In wild relatives of wheat, triosephosphate isomerase of *S. cereale* has been

Table 2. Fungal resistance genes cloned from wheat and related species.

Gene	Disease	Origin	Accession no.	Function	Reference
Lr1	leaf rust	Wheat	EF439840	While *Lr1* is not very effective in controlling leaf rust, the *Lr1/Avr1* interaction is a good example of a gene-for-gene system. The overexpression of *Lr10* resulted in enhanced resistance with a complete prevention of rust sporulation	Cloutier *et al*. (2007)
Lr10	leaf rust	*T. monococcum*	AAQ01784		Feuillet *et al*. (2003)
Lr21	leaf rust	Wheat	AY139586	*Lr21* is potentially durable and highly effective leaf rust resistance gene in wheat	Huang *et al*. (2003)
Lr21	leaf rust	*Ae. tauschii*	AAO12455		
Lr34/Yr18	leaf rust	Wheat	Pending	*Lr34/Yr18* provides durable resistance for leaf rust and stripe rust	Fu *et al*. (2009)
Mla1	powdery mildew	Barley	AAG37354	The *Mla* locus encodes a large number of characterized resistance specificities, each recognizing unique fungal determinants that are encoded by cognate fungal avirulence genes. The *Mla* locus provides a diversity of resistant phenotypes	Zhou *et al*. (2001)
Mla6	powdery mildew	Barley	CAC29242		Halterman *et al*. (2001)
Mla12	powdery mildew	Barley	AAO42441		Shen *et al*. (2003)
Mlo	powdery mildew	Barley	CAB06083	*Mlo* confers a broad spectrum resistance to almost all known isolates of the fungal pathogen	Büschges *et al*. (1997)
PM3	powdery mildew	Wheat	AAQ96158	*Pm3* is one the leucine-rich repeat type resistance genes, that confers AvrPm3b-dependent resistance to wheat powdery mildew	Yahiaoui *et al*. (2004)
Rgh1	powdery mildew	Barley	AAM22828	Resistance gene homolog (*Rgh*) containing CC, NBS, LRR domains constitute the major defense that is related to the barley *Mla* locus	Wei *et al*. (2002)
Rgh2	powdery mildew	Barley	AAM22820		
Rpg1	stem rust	Barley	ABG48657	*Rpg1* has durable protection against stem rust losses in barley, which encodes a receptor kinase-like protein with two tandem domains	Brueggeman *et al*. (2002)
Yr10	stripe rust	Wheat	AAG42168	*Yr10* belongs to the classical NBS-LRR gene family, providing race specific resistance to strip rust	(Fu, personal communication)
Yr36	stripe rust	Wheat	Pending	*Yr36* provides partial resistance to a broad range of races of stripe rust under high temperatures	Fu *et al*. (2009)

cloned (Schmidt *et al*. 1995), and it might potentially be used to meliorate the efficiency of photosynthesis.

Nutrition

The high-affinity K^+ uptake system of plants is of crucial importance in their nutrition and has been the subject of extensive kinetic studies. *HvHAK* from barley roots, conferring high-affinity K^+ uptake has been characterized, and found to also mediate low-affinity Na^+ uptake (Santa-María *et al*. 1997). Soils in many agricultural areas have high pH, resulting in low availability of zinc and iron. Plants grown on such soils suffer from either Zn or Fe deficiency or both. Iron-efficient graminaceous monocots release iron chelating substances and mugineic acid family phytosiderophores (MAs) in response to iron deficiency stress. The IDI-1 (Iron deficiency induced-1) gene and the putative gene fragment for SAM-s (S-adenosylmethionine synthetase) have already been identified in barley (Yasemin 2003). Laurie *et al*. (2002) transformed wheat with both sense and antisense constructs of high affinity potassium transporter HKT1, and characterized it with different magnitudes of salt stress suggesting its role in the roots of wheat plants and also the Na^+: K^+ ratios were reduced in salt stressed transgenic tissue when compared with controls.

Vernalization

Plants with a winter growth habit need to undergo vernalization (several weeks of cold exposure) to induce flowering. Winter wheat requires 6–8 weeks at a low temperature (2–4°C) to flower. The main genes (*VRN1* and *VRN2*), which control the vernalization process have been cloned (Yan *et al*. 2003, 2004). Loss-of-function of *VRN2*, whether by natural mutations or deletions, resulted in spring lines, which do not require vernalization to flower (Yan *et al*. 2004). Plants homozygous for the *Vrn1* allele for a spring growth habit showed no significant effects of the *VRN2* gene on flowering time. The epistatic interactions between vernalization genes *VRN1* and *VRN2* suggested a model in which *VRN2* would repress directly or indirectly the expression of *AP1*. A mutation in the promoter region of *AP1* would result in the lack of recognition of the repressor and thus inducing the dominant spring growth habit (Yan *et al*. 2003).

Considering these factors, a model for the regulation of flowering by vernalization in wheat was summarized (Yan *et al*. 2003). This model provides an explanation for the parallel evolution of *VRN1* spring alleles in three different Triticeae lineages. Most of the wild Triticeae have a winter growth habit, suggesting that the recessive *vrn1* allele is the ancestral character. The confirmation of the hypotheses generated by the model presented here will contribute to unraveling the complex set of relationships responsible for the regulation of heading date in temperate cereals.

Conclusion

Genetic transformation is vital to the transfer of novel genes into crop plants as well as to the emerging area of functional genomics (Bhalla *et al*. 2006). Wheat transformation involves the introduction of genes from foreign source, most often related to wheat. However, the successful genetic transformation of wheat still remains time consuming and genotype-dependent (Bhalla *et al*. 2006). At present, the process of genetic transformation in wheat has been successfully carried out. Transformation of wheat is usually carried out either by a gene (biolistic) gun or by the use of a bacterial vector in a dish in a laboratory. Wheat researchers agree that there appears to be a tremendous potential for wheat improvement, as biotechnology could be used to add quality, abiotic tolerance, and biotic resistance, including viral disease resistance.

Appendix

Table 1A. The seed storage protein genes cloned from wheat and its related species (data was obtained by blast in NCBI database). HMW/LWM-GSs/, high/low molecular weight glutenin subunits.

Species	HWM-GSs	LMW-GSs	α/β-Gliadin	γ-Gliadin	ω-Gliadin
Aegilops bicornis	145			4	
Ae. columnaris		1			
Ae. comosa	55	13			
Ae. crassa	12				
Ae. cylindrica	88				
Ae. geniculata		13			
Ae. juvenalis		1			
Ae. kotschyi	7				
Ae. longissima		11	89	4	
Ae. markgrafii	54	1			
Ae. searsii	152			2	
Ae. sharonensis				2	
Ae. speltoides	21	1	46	2	
Ae. tauschii	956	35	87	10	1
Ae. tauschii × *T. turgidum*		4			

Table 1A (*contd.*)

Species	HWM-GSs	LMW-GSs	α/β-Gliadin	γ-Gliadin	ω-Gliadin
Ae. triuncialis		3			
Ae. umbellulata	64	2			
Ae. uniaristata	49	2			
Ae. ventricosa	13				
Agropyron cristatum	175				
Australopyrum retrofractum			16		
Crithopsis delileana	69	2	3	2	
Dasypyrum breviaristatum	34		27		
D. villosum	30		16		
Elymus alashanicus	29				
E. sibiricus			2		
Hordeum chilense	27	20			
H. brevisubulatum ssp. *turkestanicum*		2			
H. vulgare	8	9			2
H. vulgare ssp. *vulgare*	20	16			
Leymus mollis	62				
L. multicaulis	66				
L. paboanus	44				
L. racemosus	30				
L. secalinus	27				
Pseudoroegneria spicata			11		
P. stipifolia	85				
Secale cereale	397			3	3
S. strictum				1	
S. sylvestre		2		1	
S. vavilovii				1	
synthetic construct	50				
Taeniatherum caput-medusae var. *crinitum*	63	1			
Thinopyrum bessarabicum	31				
Th. elongatum	784	25	134		
Th. intermedium	100	5	10		
Th. junceum	61				
Th. ponticum × *T. aestivum*	203	41	29		
Triticum aestivum	2074	458	135	58	4
T. aestivum ssp. *compactum*	16			2	
T. aestivum ssp. *macha*		2		3	
T. aestivum ssp. *spelta*	130		2	4	
T. aestivum ssp. *sphaerococcum*			21	2	
T. aestivum ssp. *tibeticum*	17	3			
T. aestivum ssp. *yunnanens*	18				
T. aestivum var. *arduini*			1		
T. aestivum × *Th. elongatum*		3			
T. aestivum/Th. intermedium addition line		5			
T. monococcum		18	57		
T. monococcum ssp. *aegilopoides*		6	5		
T. timopheevii	104		1	4	
T. turgidum	105	11			
T. turgidum ssp. *dicoccoides*	78	8	6	4	
T. turgidum ssp. *dicoccon*	42			3	
T. turgidum ssp. *durum*	60	11	5	12	
T. turgidum ssp. *durum* × *H. chilense*				1	
T. turgidum ssp. *paleocolchicum*			13		
T. turgidum ssp. *polonicum*	54	2			
T. urartu	55	2	4		
T. vavilovii				2	
T. zhukovskyi		5	1		

Table 1B. Part of known gliadin genes from wheat and *Aegilops*.

Type	Number	Origin	Length (bp)
α-Type	AJ133612	*Triticum aestivum*	873
	AY293730		1335
	CV066588		753
	D84341		780
	K02068		1152
	M11075		1126
	X17361		994
	X54688		3573
	AJ130948	*T. aestivum* ssp. *spelta*	1081
	DQ234067		846
	DQ296197	*T. turgidum* ssp. *durum*	849
	DQ002583	*T. monococcum*	807
	DQ002588	*A. speltoides*	810
	DQ002599	*A. tauschii*	801
	DQ002736	*A. longissima*	783
γ-Type	AF144104	*T. aestivum*	956
	AJ416339		840
	EF151018		1142
	M11076		1039
	M13713		2450
	M16060		798
	M16064		1397
	M36999		2086
	AF120267	*T. aestivum* ssp. *spelta*	947
	AJ389670	*T. aestivum* ssp. *compactum*	850
	AJ389673	*T. aestivum* ssp. *macha*	801
	AJ389676	*T. aestivum* ssp. *sphaerococcum*	801
	AJ389678	*T. vavilovii*	762
	AJ389710	*T. timopheevii*	900
	AJ389722	*T. urartu*	577
	AJ389708	*T. turgidum* ssp. *dicoccoides*	949
	AY338394	*T. turgidum* ssp. *durum*	729
	X53412		842
	X77963		906
	AJ389688	*A. tauschii*	607
	AJ389712	*A. bicornis*	902
	AJ389714	*A. longissima*	883
	AJ389715	*A. searsii*	694
	AJ389718	*A. sharonensis*	704
	AJ389720	*A. speltoides*	771
ω-Gliadin	AB059812	*T. aestivum*	1858
	AB181300		1413
	AB181301		1275
	AF280605		3789
	AF280606		3925
	AJ937839		1399
	AY591334		1440
	DQ287981		1080
	DQ307378		1080
	DQ317535		1299
	DQ861428	*T. timopheevii*	1004
	AY667097	*A. tauschii*	1216

Table 1C. Part of HMW-GS genes.

Accession	Source/species	Protein	Genome[1]
AY611726	*A. bicornis*	Sby2.0	Sb
AY611728	*A. bicornis*	Sby2.5	Sb
AY455788	*A. comosa*	My	M
AJ306973	*A. cylindrical*	Cy	CD
AJ306974	*A. cylindrical*	Dy	CD
AY303127	*A. kotschyi*	Svy	USv
AY303126	*A. kotschyi*	Uy	USv
AF476960	*A. markgrafii*	Cy	C
AY611724	*A. searsii*	Ssy2.0	Ss
AY611721	*A. searsii*	Ssy2.1	Ss
AF513640	*A. speltoides*	Sy	S
AY594358	*A. tauschii*	Dy10t	D
AY248704	*A. tauschii*	Dy12.1t	D
U39229	*A. tauschii*	Dy12t	D
AF476962	*A. umbellulata*	Uy	U
AY455787	*A. uniaristata*	Ny	N(Mu)
AF226698	*A. ventricosa*	y-type	DM
AY834230	*C. delileana*	Ky	K
AY608740	*D. villosum*	Vy	V
AY299518	*E. elongata*	Eey1.8	Ee
AY298724	*E. elongata*	Eey1.5	Ee
AJ314767	*S. cereale*	Ry	R
AJ314770	*S. cereale*	Ry	R
AY303125	*T. caput-medusae*	T_ay	T_a
X03042	*T. aestivum*	Ay	ABD
X61026	*T. aestivum*	By9	ABD
X03041	*T. aestivum*	Dy12	ABD
X12929	*T. aestivum*	Dy10	ABD
DQ086215	*T. aestivum*	By15	ABD
AY321449	*T. aestivum* ssp. *compactum*	y-type	ABD
AJ566642	*T. aestivum* ssp. *spelta*	Ay2	ABD
AY321450	*T. aestivum* ssp. *spelta*	y-type	ABD
AY695379	*T. petropavlovskyi*	Dy10.1	ABD
AJ306977	*T. timopheevii*	Ay	AG
AY321445	*T. turgidum* ssp. *dicoccum*	By22*	AB
AY245797	*T. turgidum* ssp. *durum*	By8	AB
AY722710	*T. turgidum* ssp. *polonicum*	Ay	AB
AY245578	*T. urartu*	Ay-Tu-e	Au
AY245579	*T. urartu*	Ay-Tu-s	Au
AY611725	*A. bicornis*	Sbx2.5	Sb
AY611727	*A. bicornis*	Sbx3.0	Sb
AY455789	*A. comosa*	Mx	M
AJ306975	*A. cylindrical*	Dx	CD
AF476959	*A. markgrafii*	Cx	C
AY611723	*A. searsii*	Ssx2.4	Ss
AY611722	*A. searsii*	Ssx2.5	Ss
AF480486	*A. tauschii*	Dx2.1t	D
AF480485	*A. tauschii*	Dx2t	D
AY804129	*A. tauschii*	Dx5t	D
AF476961	*A. umbellulata*	Ux	U
AY455786	*A. uniaristata*	Nx	N(Mu)
AF226699	*A. ventricosa*	x-type	DM
AY804128	*C. delileana*	Kx	K
AY525782	*E. bessarabicum*	Ebx	Eb
AY299519	*E. elongata3*	Eex2.1	Ee
AY299520	*E. elongata*	Eex1.9	Ee

Table 1C (*contd.*)

Accession	Source/species	Protein	Genome[1]
AJ314768	*S. cereale*	Rx	R
AJ314769	*S. cereale*	Rx	R
AY299654	*T. caput-medusae*	T_ax	T_a
X61009	*T. aestivum*	Ax1	ABD
M22208	*T. aestivum*	Ax2*	ABD
AF145590	*T. aestivum*	Ax-null	ABD
AY367771	*T. aestivum*	Bx14	ABD
AY553933	*T. aestivum*	Bx23	ABD
X13927	*T. aestivum*	Bx7	ABD
X03346	*T. aestivum*	Dx2	ABD
AY159367	*T. aestivum*	Dx2.2	ABD
X12928	*T. aestivum*	Dx5	ABD
AJ567923	*T. aestivum* ssp. *spelta*	Bx	ABD
AY321452	*T. aestivum* ssp. *spelta*	Bx6.1	ABD
AY517724	*T. petropavlovskyi*	Dx2.1	ABD
AJ306976	*T. timopheevii*	Ax	AG
AY494981	*T. turgidum*	Ax	AB
AY621068	*T. turgidum* ssp. *dicoccoides*	Bx	AB
AY848709	*T. turgidum* ssp. *dicoccoides*	Bx	AB
AJ437000	*T. turgidum* ssp. *durum*	Bx20	AB
AY722709	*T. turgidum* ssp. *polonicum*	Bx7.1	AB

[1] The nuclear genome symbols in the tribe *Triticeae* is proposed by Hsiao *et al*. (1995).

Table 1D. Part of known LMW-GS genes from common wheat.

Accession	Origin	Location
AY299485	Shaanyou 225 (*T.aestivum L.*)	*Glu-D3*
AY263369	Xianyan 6 (*T.aestivum L.*)	*Glu-D3*
AB062875	Norin 61 (*T. aestivum L.*)	*Glu-D3*
AB062872	Norin 61 (*T. aestivum L.*)	*Glu-D3*
AB062867	Norin 61 (*T. aestivum L.*)	*Glu-D3*
AB062866	Norin 61 (*T. aestivum L.*)	*Glu-D3*
AB062865	Norin 61 (*T. aestivum L.*)	*Glu-D3*
AB062851	Norin 61 (*T. aestivum L.*)	*Glu-D3*
X84961	Chinese Spring (*T. aestivum L.*)	*Glu-D3*
AB062865	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062864	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062863	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062862	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062861	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062860	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062859	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062858	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062857	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062856	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062855	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062854	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062853	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AB062852	Norin 61 (*T. aestivum L.*)	*Glu-B3*
AY007746	*T. turgidum* ssp. *durum* Line 21	*Glu-B3*
Y17845	Yecora Rojo (*T. aestivum L.*)	*Glu-B3*
Y14104	*T. turgidum* ssp. *durum* Langdon	*Glu-B3*
X84960	Chinese Spring (*T. aestivum L.*)	*Glu-B3*
AB062878	Norin 61 (*T. aestivum L.*)	*Glu-A3*
AB062877	Norin 61 (*T. aestivum L.*)	*Glu-A3*

Table 1D (*contd.*)

Accession	Origin	*Location*
AB062876	Norin 61 (*T. aestivum L.*)	*Glu-A3*
AB062871	Norin 61 (*T. aestivum L.*)	*Glu-A3*
AB062870	Norin 61 (*T. aestivum L.*)	*Glu-A3*
AB062869	Norin 61 (*T. aestivum L.*)	*Glu-A3*
AB062868	Norin 61 (*T. aestivum L.*)	*Glu-A3*
AJ293099	*T. turgidum* ssp. *durum* Langdon	*Glu-A3*
AJ293098	*T. turgidum* ssp. *durum* Langdon	*Glu-A3*
AJ293097	*T. turgidum* ssp. *durum* Langdon	*Glu-A3*
AY453160	Glenlea (*T. aestivum L.*)	*Glu-A3*
AY453159	Rescue (*T. aestivum L.*)	*Glu-A3*
AY453158	Halberd (*T. aestivum L.*)	*Glu-A3*
AY453157	Suneca (*T. aestivum L.*)	*Glu-A3*
AY453156	Cheyenne (*T. aestivum L.*)	*Glu-A3*
AY453155	Gabo (*T. aestivum L.*)	*Glu-A3*
AY453154	Chinese Spring (*T. aestivum L.*)	*Glu-A3*
AY146588	*T. monococcum*	*Glu-A3*
X84959	Chinese Spring (*T. aestivum L.*)	*Glu-A3*

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