Theor Appl Genet (2012) 125:1–10
DOI 10.1007/s00122-012-1829-3

REVIEW

Functional markers in wheat: current status and future prospects

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Received: 4 October 2011 / Accepted: 11 February 2012 / Published online: 26 February 2012

Abstract Functional markers (FM) are developed from sequence polymorphisms present in allelic variants of a functional gene at a locus. FMs accurately discriminate alleles of a targeted gene, and are ideal molecular markers for marker-assisted selection in wheat breeding. In this paper, we summarize FMs developed and used in common wheat. To date, more than 30 wheat loci associated with processing quality, agronomic traits, and disease resistance, have been cloned, and 97 FMs were developed to identify 93 alleles based on the sequences of those genes. A general approach is described for isolation of wheat genes and development of FMs based on in silico cloning and comparative genomics. The divergence of DNA sequences of different alleles that affect gene function is summarized. In addition, 14 molecular markers specific for alien genes introduced from common wheat relatives were also described. This paper provides updated information on all FMs and gene-specific STS markers developed so far in wheat and should facilitate their application in wheat breeding programs.

Communicated by R. Varshney.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-012-1829-3) contains supplementary material, which is available to authorized users.

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Introduction

Functional or gene-specific markers are derived from polymorphic sites within genes that are directly associated with phenotypic variations (Andersen and Lübberstedt 2003; Gupta and Rustgi 2004; Bagge et al. 2007). During the past decades, molecular mapping has identified chromosome regions carrying important genes in wheat using SSR, RFLP, AFLP, RAPD and DArT markers. However, these usually neutral genetic markers can be some distance from the targeted genes, and thus are often population-specific or parent related, and their predictive value depends on the degree of linkage between markers and target locus alleles in specific populations (Bagge et al. 2007). As a result, relatively few linked markers are used in breeding. In contrast, FMs developed from functional gene sequences accurately discriminate alleles at one locus and represent ideal markers for marker-assisted selection (MAS) in breeding. FMs have apparent advantages over random DNA markers, because they are diagnostic of the desired trait allele (Varshney et al. 2005).

In plant breeding, FMs can be used for validation of cultivar identity, selection of parental materials to build segregating population, and subsequent selection of lines (Lübberstedt et al. 2005). Bagge et al. (2007) described the development and application of FMs in wheat. Nevertheless, many new wheat genes have been cloned during the

past years, and the corresponding FMs have been developed and used in wheat breeding. To date, more than 30 loci (genes) have been cloned in common wheat and its relatives, and 97 FMs for wheat processing quality, agronomic traits and disease resistance genes have been developed and used to identify 93 alleles (Sup-Tables 1, 2 and 3).

The objectives of this paper were to (1) summarize the FMs in wheat and gene-specific markers for the genes introduced from alien species, (2) describe a general method for isolation of wheat genes and development of FMs using in silico cloning approach, and (3) discuss a prospect of potential high-throughput marker technology in wheat breeding.

Development of FMs for genes from wheat genome

FMs were developed from single nucleotide polymorphisms (SNPs) or insertions/deletions (InDels) between different alleles, requiring the gene sequences of functional motifs associated with plant phenotypes. Hence isolating genes and finding functional motifs within genes associated with plant phenotypes are essential for FM development (Bagge et al. 2007).

Map-based cloning is an effective method to isolate genes in plants (Yan et al. 2004b). Using this approach, around ten wheat genes were cloned during the past decade (Sup-Tables 2 and 3). However, common wheat has a very large genome, and the genomic sequences are not yet assembled as thoroughly as those in rice and maize. It is therefore very difficult to clone genes by map-based cloning in common wheat.

Alternatively, comparative genomics provides an efficient approach for isolation of wheat genes. Orthologs descended from a common ancestor often have conserved functions and are expected to produce similar phenotypes across species (Devos 2005). The grass genomes of rice, maize and *Brachypodium* have been sequenced and provided powerful tools for gene discovery in wheat (Matsumoto et al. 2005; Schnable et al. 2009; Vogel et al. 2010). The technology for in silico cloning is widely employed for identifying genes of interest in wheat (Gill and Sanseau 2000; He et al. 2007, 2008, 2009a; Su et al. 2011; Ma et al. 2012). Based on the expressed sequence tags (EST) database, the putative wheat gene sequences were obtained by aligning and jointing the orthologous genes with the same function in grass. The procedures of in silico cloning gene and functional marker development are shown in Fig. 1. Based on the cDNA sequence of maize *Psy1* gene (GenBank accession U32636), all wheat ESTs sharing high similarity with the reference gene were blasted and subjected to contig assembly (He et al. 2008). The wheat *Psy1* gene was cloned with PCR amplification, and an FM YP7A for discrimination of two alleles at *Psy-A1* locus was developed and validated using 217 Chinese wheat cultivars and 240 $F_{5:6}$ lines from the cross of PH82-2/Neixiang 188.

Functional markers for processing quality traits

The processing quality of wheat-based products is highly associated with high- and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS), polyphenol oxidase (PPO) activity, lipoxynase (LOX) activity, yellow pigment content (YPC), kernel hardness, and starch properties. In total, 16 loci with 62 alleles for these traits have been cloned and 56 FMs have been developed (Sup-Table 1).

Polyphenol oxidase (PPO) activity is a vital factor causing undesirable brown discoloration of the end-use products of wheat, particularly Asian noodles. In order to screen cultivars with low PPO activity, markers were developed based on allelic variants of PPO genes on chromosomes 2A and 2D (Sun et al. 2005; He et al. 2007). The functional markers PPO18 and PPO33 for *Ppo-A1* amplify 87 and 481 bp PCR fragments, respectively, in cultivars with lower PPO activity, whereas PPO16 and PPO29 discriminate alleles *Ppo-D1a* and *Ppo-D1b*, associated with lower and higher PPO activity, respectively (Sup-Table 1). These FMs can be efficiently used to identify wheat lines with lower PPO activity (Xiao et al. 2008; Singh et al. 2009; Liang et al. 2010). Nevertheless, a functional marker for the PPO gene on chromosome 2B is currently not available due to lack of polymorphisms at this locus in Chinese wheats even though the gene has been sequenced (our unpublished data).

LOX activity also has a major effect on color and processing quality of wheat-based products (Geng et al. 2012). Functional markers LOX16 and LOX18 for *TaLox-B1*, a LOX gene located on chromosome 4BS, were developed, and they amplify 48 and 791 bp PCR fragments in cultivars with higher and lower LOX activities, respectively (Sup-Table 1). The two FMs were developed from a SNP in the third exon of *TaLox-B1a* and *TaLox-B1b*, and PCR conditions should be optimized prior to use. Yellow pigment content is closely related to the color of wheat-based products. A bright white color is preferred for Chinese white salted noodles, whereas yellow alkaline noodles with bright yellow color are widely preferred in southeastern Asia and Japan (Kruger et al. 1992; Parker et al. 1998). Carotenoids are the main component of wheat flour yellow pigment (Miskelly 1984; Howitt and Pogson 2006; He et al. 2008). Phytoene synthase (PSY) and ζ (zeta)-carotene desaturase (ZDS) are critical enzymes in the biosynthetic pathway for carotenoid synthesis in wheat (He et al. 2008; Zhang et al. 2011; Dong et al. 2012). Functional markers

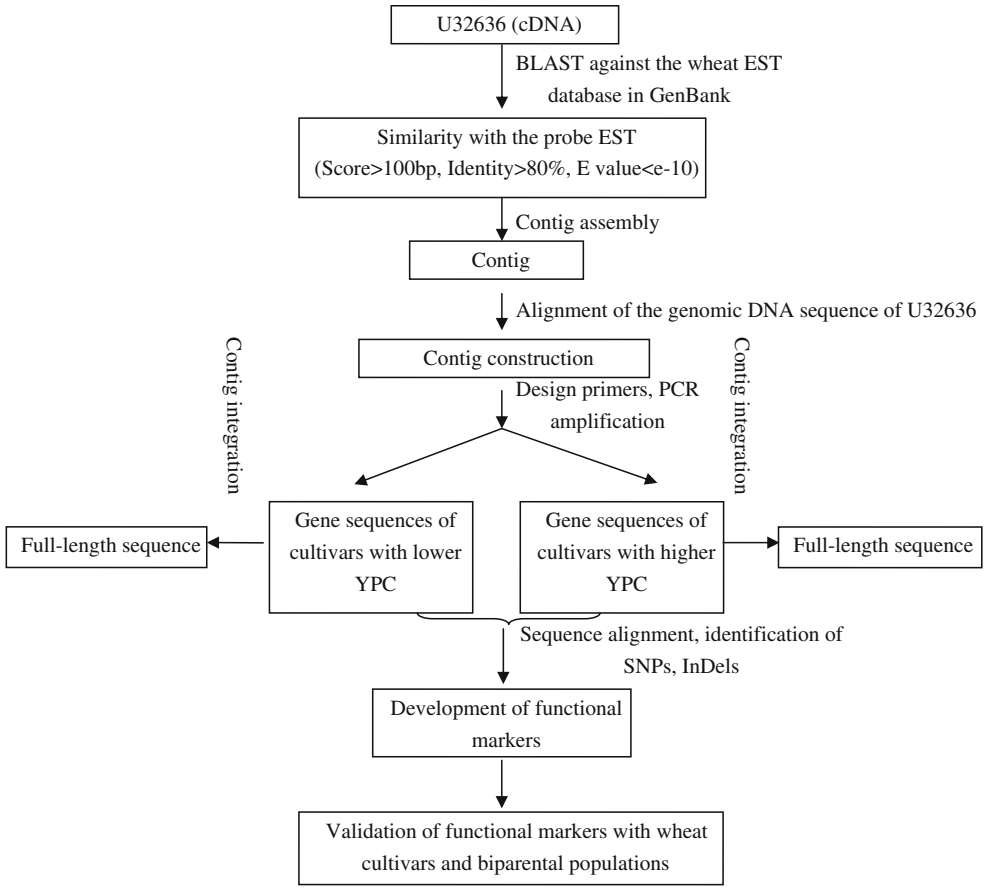

Fig. 1 The procedure for cloning *Psy1* gene in common wheat and development of functional markers

are available for PSY genes on chromosomes 7AL, 7BL and 7DL (He et al. 2008, 2009a; Wang et al. 2009a), and for ZDS genes on chromosomes 2A and 2D (Zhang et al. 2011; Dong et al. 2012) (Sup-Table 1), and they accurately discriminate different alleles in wheat.

HMW-GS and LMW-GS are the most important storage proteins of wheat. They are encoded by the *Glu-1* and *Glu-3* loci, respectively, on wheat homoeologous group 1 chromosomes, and play important roles in determining dough quality as they confer viscoelasticity and extensibility properties required for mixing and baking performance in common wheat (Luo et al. 2001). According to the nucleotide sequences of cloned genes, functional markers were developed to identify alleles for HMW-GS and LMW-GS in wheat cultivars (D'Ovidio and Anderson 1994; Smith et al. 1994; Ma et al. 2003; Schwarz et al. 2004; Lei et al. 2006; Ishikawa and Nakamura 2007; Liu et al. 2008b; Ragupathy et al. 2008; Wang et al. 2009b, 2010; Sup-Table 1). Markers for *Glu-A3* alleles were based on DNA polymorphisms identified between the LMW glutenin genes (Zhang et al. 2004), and markers developed by Wang et al. (2009b, 2010) are much easier to use. However, no functional markers for the *Glu-D3* locus were developed due to the very small variations among alleles (Liu et al. 2010), but its impact on dough quality is relatively small in comparison with *Glu-A3* and *Glu-B3* loci (Gupta et al. 1989). A total of 7 and 10 FMs were available for *Glu-A3* and *Glu-B3* loci, respectively, and multiplex PCRs were established for these FMs to increase the efficiency of marker selection (Wang et al. 2009b, 2010). The FMs for HMW-GS can be efficiently used to test wheat cultivars and lines (Liang et al. 2010; Jin et al. 2011; Ram et al. 2011).

Kernel hardness, which has a profound effect on milling and end-use quality, is largely determined by the *Pina-D1* and *Pinb-D1* genes encoding puroindoline a and puroindoline b proteins, respectively. Functional markers were designed for identifying the genotypes at *Pina-D1* and *Pinb-D1* loci (Giroux and Morris 1997; Gazza et al. 2005; Chen et al. 2012). The FM for *Pinb-D1b* associated with superior milling and processing qualities is most useful in wheat breeding (Chen et al. 2012).

The GBSS I (granule-bound starch synthase) gene product is known as Waxy (Wx) protein, and it is a key

enzyme for amylose synthesis in the endosperm. Amylose content in wheat plays a significant role in determining noodle quality. High noodle-making quality of wheat cultivars without Wx-B1 protein that is controlled by Wx-B1 null allele had low amylose level. The functional markers were designed for identifying this allele (Saito et al. 2009; Nakamura et al. 2002). Eight isogenic wheat lines that have different combinations of presence and absence of three Wx proteins, Wx-A1, Wx-B1, and Wx-D1, were produced in order to elucidate the effect of Wx protein deficiencies on the apparent amylose content and starch properties (Yamamori and Quynh 2000). Amylose content and pasting properties of starch were determined to be influenced most by the lack of the Wx-B1 protein, followed by a lack of Wx-D1, and last by the Wx-A1 deficiency, which indicated the different effects of the three null alleles for the Wx protein (Yamamori and Quynh 2000). The genotypes with the null Wx-A1 and null Wx-D1 alleles are rarely present in commercial wheat cultivars, thus the FMs for them are less useful than those for null Wx-B1 allele.

Functional markers for agronomic traits

To date, 12 loci with 22 alleles for important agronomic traits, such as plant height, photoperiod response, grain weight, and tolerance to abiotic stress have been cloned, and 27 FMs were developed and reportedly used in wheat breeding programs (Sup-Table 2).

The semi-dwarfing genes *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) widely present in commercial cultivars reduce plant height, increase harvest index, and improve lodging resistance, and consequently increase grain yield. Functional markers for the two genes were developed and used for discrimination of semi-dwarf (*Rht-B1b* and *Rht-D1b*) and wild-type alleles (*Rht-B1a* and *Rht-D1a*) (Ellis et al. 2002; Zhang et al. 2006). However, the PCR conditions are very stringent for the FMs of *Rht-B1* and *Rht-D1*, in which Hotstar *Taq* DNA polymerase needs to be used for a reliable PCR amplification (Ellis et al. 2002). This will limit the use of these markers in breeding due to high costs (Bagge and Lübberstedt 2008).

Adaptation of common wheat cultivars to diverse environmental conditions is greatly influenced by flowering time, mainly determined by three groups of genes, viz. photoperiod response genes (*Ppd*), vernalization genes (*Vrn*) and developmental rate genes (Yang et al. 2009). The dominant alleles (*Ppd-A1a*, *Ppd-B1a* and *Ppd-D1a*), located on the short arms of chromosomes 2A, 2B and 2D, respectively, confer photoperiod insensitivity, whereas the recessive alleles (*Ppd-A1b, Ppd-B1b* and *Ppd-D1b*) confer photoperiod sensitivity, and *Ppd-D1a* is the major photoperiod insensitivity allele in common wheat (Beales et al. 2007). Functional markers for *Ppd-D1* were developed (Beales et al. 2007) and used to test the adaptation and productivity of Chinese wheat cultivars (Yang et al. 2009). Common wheat is divided into spring and winter types based on growth habits that are determined by vernalization response genes, located on homoeologous group 5 chromosomes, and chromosome 7B. Functional markers are available (Sup-Table 2) to detect the distribution of growth habit in cultivars and to determine the relationship between growth habit and combinations of *Vrn* alleles, which directly affect heading date phenotype (Yan et al. 2004a, 2006; Fu et al. 2005; Zhang et al. 2008b; Chu et al. 2011).

Thousand kernel weight (TKW) is an important component of grain yield in wheat and is conditioned by polygenes. Sucrose synthase 2 (Sus2), grain width (GW2) and cell-wall invertase (CWI) genes are associated with TKW in rice (Huang et al. 1996; Song et al. 2007; Wang et al. 2008). These genes were cloned in common wheat using in silico cloning approaches, and the corresponding functional markers were developed and used to test Chinese wheat cultivars (Jiang et al. 2011; Su et al. 2011; Ma et al. 2012; Sup-Table 2). Jiang et al. (2011) reported that the Hap-H and Hap-L haplotypes were significantly associated with TKW in 89 modern wheat cultivars from Chinese mini-core collection, and haplotype Hap-H had a significant positive effect on TKW. Association analysis indicated that Hap-6A-A haplotype at *TaGW2-6A* locus not only increased TKW but also is associated with earlier heading and maturity (Su et al. 2011; Sup-Table 2). A total of 265 Chinese wheat cultivars were genotyped and association analysis revealed the Hap-6A-A was significantly associated with wider grains and higher TKW in two cropping seasons, and a CAPS marker permitted generation of different *Taq*I restriction fragments, producing 167 and 218 bp PCR fragments in the cultivars with Hap-6A-A and Hap-6A-G, respectively (Su et al. 2011; Sup-Table 2). Two sets of Chinese landraces and two sets of commercial wheat cultivars were used to validate the association of functional markers CWI21 (*Tacwi-A1b*) and CWI22 (*Tacwi-A1a*) with kernel weight, and their amplified PCR products are associated with lower and higher TKW, respectively (Ma et al. 2012). Dehydration-responsive element binding (DREB) proteins are induced by abiotic stresses. A DREB gene was induced by low temperature, drought and ABA treatment. Based on the allelic variations at *Dreb-B1* locus on chromosome 3BL, a functional marker was designed for improving drought tolerance in wheat (Wei et al. 2009).

Compared with quality traits, the agronomic traits are much more easy to be characterized, and can be directly selected based on their performance in the field, thus the FMs for agronomic traits are less useful than those for quality traits in wheat breeding.

Functional markers for disease resistance genes

During the past decade, six genes for disease resistance were cloned in wheat (Laroche et al. 2000; Feuillet et al. 2003; Huang et al. 2003; Yahiaoui et al. 2004; Fu et al. 2009; Krattinger et al. 2009). Among them, functional markers are available for alleles at the *Pm3* locus for reaction to powdery mildew and for the *Lr34/Yr18/Pm38* locus for resistance to leaf rust, stripe rust and powdery mildew (Tommasini et al. 2006; Lagudah et al. 2009, Sup-Table 3). According to the nucleotide polymorphisms of coding sequences and adjacent untranslated regions of *Pm3* resistance alleles, seven allele-specific molecular markers were designed, and successfully discriminated allelic variants at the *Pm3* locus (Tommasini et al. 2006). These markers were used to identify desirable alleles in the US and European wheat cultivars (Peusha et al. 2008; Chen et al. 2009; Lillemo et al. 2010; Mohler et al. 2011), but they are not used in Chinese wheat breeding because all the seven *Pm3* alleles are susceptible to the prevalent Chinese *Blumeria graminis* f. sp. *tritici* races. The *Lr34/Yr18/Pm38* locus confers durable partial adult-plant resistance to multiple diseases, and is used in wheat breeding programs worldwide. The functional markers for *Lr34/Yr18/Pm38* can easily be resolved in standard agarose gels, providing a simple and efficient tool to detect the presence or absence of the resistance gene (Lagudah et al. 2009). When 273 CIMMYT wheat lines were tested with *Lr34/Yr18/Pm38* markers, a very good association between the marker data and stripe rust severities were obtained, indicating the markers are very effective (Wu et al. 2010). However, in a test of 151 Chinese wheat landraces using the *Lr34/Yr18/Pm38* marker, 82.1% possessed the *+Lr34/Yr18/Pm38* allele, but one-quarter of that group was highly susceptible to stripe rust in the field (Wu et al. 2010). DNA sequencing indicated that the allele in these landraces showed the same sequence as the *+Lr34/Yr18/Pm38* allele (our unpublished data). Susceptibility to stripe rust and leaf rust in this group may be due to the presence of inhibitor genes or absence of a functional gene that is essential in the biosynthetic pathway for the expression of *Lr34/Yr18/Pm38*.

Gene-specific markers for the genes introduced from alien species

For centuries breeders have made great efforts to introduce resistance genes from wheat relatives into cultivated wheat cultivars. Wheat wild relatives are important germplasm resources to supply new genes to reduce fungicide applications in wheat production. Molecular markers specific to the transferred alien chromosomal segments were available for these genes (Sup-Table 4).

The 1B·1R translocation has been widely used in global wheat breeding programs. It has positive effects on grain yield, adaptation, and other agronomic traits although its resistances to diseases have been overcome in many locations. However, the 1B·1R translocation has significantly negative effects on dough qualities for both bread and Chinese noodles (He et al. 2005). Hence, it is important to identify the 1B·1R translocation in wheat breeding. Functional markers based on the rye secalin gene on 1RS were successfully applied in breeding (Froidmont 1998; Chai et al. 2006; Liu et al. 2008a; Sup-Table 4).

Stripe rust resistance gene *Yr17* from 2NS of *Triticum ventricosum* Tausch has been translocated to the short arm of bread wheat chromosome 2AS (Helguera et al. 2003), and this chromosomal segment also conferred resistance to leaf rust (*Lr37*) and stem rust (*Sr38*). The *Lr19* gene, originated from decaploid *Thinopyrum ponticum*, was transferred into durum wheat, and widely conferring resistance to leaf rust in wheat (Gennaro et al. 2009). *Ae. tauschii* Cosson was the donor of the *Lr21* that is a durable and highly effective leaf rust resistance gene, and it has been incorporated into wheat cultivar and is available for breeding (Talbert et al. 1994). The leaf rust resistance gene *Lr47* confers resistance to a wide spectrum of leaf rust strains. This gene was recently transferred from chromosome 7S of *Triticum speltoides* Tausch to chromosome 7A of common wheat (Helguera et al. 2000). Leaf rust resistance gene *Lr51*, located within a segment of *Triticum speltoides* Tausch chromosome 1S, was translocated to the long arm of chromosome 1B of bread wheat, which is resistant to the current predominant races in USA (Helguera et al. 2005). The gene-specific markers *Xucw108* and *Xuhw89* for *Gpc-B1* and *Yr36* originated from chromosome 6BS of *Triticum turgidum* ssp. *dicoccoides*. They were identified and validated in a collection of 117 cultivated tetraploid and hexaploid wheat germplasm (Distelfeld et al. 2006; Distelfeld and Fahima 2007; Uauy et al. 2006;Brevis and Dubcovsky 2008).

Application of functional markers in wheat breeding

Currently, FMs are widely used to test wheat cultivars and breeding lines. PPO18 and PPO33 for *Ppo-A1*, and PPO16 and PPO29 for *Ppo-D1* were reliable for evaluating the association between PPO activities and allelic variants (Sun et al. 2005; He et al. 2007). Three hundred and eleven Chinese wheat cultivars and advanced lines, 57 Indian wheat cultivars, and 273 CIMMYT wheat lines were tested with these FMs (Xiao et al. 2008; Singh et al. 2009; Liang et al. 2010).

The FM YP7A for *Psy-A1* associated with grain YP content could be efficiently used in wheat breeding (He

et al. 2008). In a test of 217 Chinese winter wheat cultivars using *YP7A*, the frequencies of *Psy-A1a* and *Psy-A1b* were 62.2 and 37.8%, respectively (Yang et al. 2008). One hundred CIMMYT durum wheat lines with widely variable grain yellowness were genotyped with the FMs YP7A, YP7A-2, YP7B-1 and YP7B-4 (He et al. 2009b). A total of 198 Chinese wheat cultivars and advanced lines were tested with the FM for *TaLox-B1*, and two allelic variants *TaLox-B1a* and *TaLox-B1b* showed highly significant association with LOX activities (Geng et al. 2012).

FMs for HMW-GS and LMW-GS were accurate and stable in the characterization of 273 CIMMYT wheat lines (Liang et al. 2010), 182 Indian wheat cultivars (Ram et al. 2011) and 718 wheat cultivars and advanced lines from 20 countries (Jin et al. 2011). The results were consistent with those tested by SDS-PAGE, indicating that these markers can be efficiently used in markers-assisted breeding.

A total of 926 Chinese wheat landraces and improved cultivars collected from nine wheat zones were characterized for their genotypes at the *Ppd-D1* locus using FMs (Yang et al. 2009). Two hundred and seventy-eight Chinese wheat cultivars were tested with FMs for the vernalization genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn-B3* (Zhang et al. 2008b). In addition, molecular markers were used to detect the presence of *Rht-B1b* and *Rht-D1b* in 220 wheat genotypes from autumn-sown wheat regions in China, which include landmark landraces, leading cultivars and core parents involved in wheat breeding from the 1950s (Zhang et al. 2006), indicating that *Rht-B1b* and *Rht-D1b* were successfully used in Chinese wheat production, with frequencies of 24.5 and 45.5%, respectively, in these cultivars.

FMs for disease resistance locus *Lr34/Yr18/Pm38* were used to test CIMMYT and Chinese wheat cultivars (Wu et al. 2010). FMs for *Pm3* locus were employed to identify different alleles in the US and European wheat cultivars (Peusha et al. 2008; Chen et al. 2009; Lillemo et al. 2010; Mohler et al. 2011).

Using the gene-specific markers *Xucw108* and *Xuhw89* for *Gpc-B1* and *Yr36*, conferring high protein content and stripe rust resistance, respectively, a hard red winter wheat cultivar Farnum (WA7975) was developed in Pullman, and a durum wheat cultivar Westmore was also commercially available to US wheat growers (Distelfeld and Fahima 2007; Brevis and Dubcovsky 2008). In addition, over 20 advanced wheat lines have been developed in China by FM-assisted selection targeting for HMW-GS, LMW-GS, PPO and PSY genes.

Knowledge of marker-trait association is a prerequisite for marker-assisted selection (Gupta et al. 2010). SNPs and InDels are the most abundant forms of DNA sequence variation in common wheat and its relatives (Ravel et al. 2006; Varshney et al. 2007). This was confirmed with cloned genes and amplicons in wheat and rye (Yahiaoui et al. 2004; Yan et al. 2006; He et al. 2007, 2008; Varshney et al. 2007; Krattinger et al. 2009; Su et al. 2011; Ma et al. 2012). SNPs and InDels among different alleles at one locus resulted in phenotypic variations in wheat (Yahiaoui et al. 2004; Yan et al. 2006; He et al. 2007, 2008; Fu et al. 2009; Gupta et al. 2009; Krattinger et al. 2009; Su et al. 2011; Ma et al. 2012). FMs can be developed based on the SNPs and InDels among different alleles at one locus, and used for marker-assisted selection.

The sequence of *TaGW2* (Su et al. 2011) was completely conserved in the coding region among cultivars, and two SNPs in the promoter region influenced both grain width and weight. A functional marker was developed based on an A–G polymorphism in the promoter region to distinguish two alleles at *TaGW2-6A* locus. qRT-PCR revealed a negative relationship between *TaGW2* expression level and grain width (Su et al. 2011).

Introns can influence gene transcription through alternative splicing (Fedorova and Fedorov 2003). Alternative splicing (AS) of pre-mRNA plays an important role in transcriptional regulation as a cause of diversity in gene expression (Wang and Brendel 2006; Reddy 2007). A typical structure for intron splicing is the consensus GT–AG structure in wheat. A functional marker PPO18 was developed based on a 191-bp InDel in the first intron of *Ppo-A1* (Sun et al. 2005). The expression of the *Ppo-A1b* allele was regulated by AS of pre-mRNAs, resulting from a 191-bp insertion in the first intron and a C/G SNP in exon II (Sun et al. 2011). Based on cDNA sequencing data, eight kinds of cDNA fragments from mature mRNA were found in the *Ppo-A1b* genotype, and seven spliced isoforms with premature termination codons resulted in potential nonsense-mediated mRNA decay. Differences in the expression of *Ppo-A1a* and *Ppo-A1b* were confirmed by PPO activity assays and whole grain staining, providing direct evidence for the influence of AS in the coding region of *Ppo-A1* on polyphenol oxidase activity in common wheat grains (Sun et al. 2011).

Segmental duplication of genes can generate new functions and expression patterns. The HMW-GS $Bx7^{OE}$, causing overexpression of Bx7 subunit in certain wheat cultivars, has a 10.3-kb segmental duplication of Bx7 gene and an LTR retroelement. Markers flanking the LTR retrotransposon borders and the duplicated region were designed at the left and right junctions of the retroelement. $Bx7^{OE}$ and Bx7 subunits can be screened by markers developed from the $Bx7^{OE}$ gene sequence (Ragupathy et al. 2008).

Wheat FMs for the future

Large-scale genome sequencing and associated bioinformatics are becoming widely accepted research tools for

accelerating the analysis of wheat genome structure and function. Second-generation DNA sequences from Chinese Spring, *Ae. tauschii* Cosson and *T. urartu* Tumanian ex Gandilyan, provide an opportunity to use genomic information to clone genes and develop SNP markers in wheat. Rapid progress is now being achieved in assembling the DNA sequences from individual chromosome arms of Chinese Spring (http://www.wheatgenome.org/) and this progress provides a template for defining the FMs for future use.

High-quality wheat genome sequences integrated with molecular genetic maps provide the basis for identifying duplicated genes, analyzing promoter regions in detail, defining SNPs/InDels and aligning the transcriptome with the genome. These advances will allow gene networks to be clearly defined and thus allow meaningful FMs to be developed for complex traits.

Extensive proteomic studies have allowed identification of many allelic variants at the *Glu-3* loci for LMW-GS, and genomic analyses identified several markers for discriminating alleles at one locus. These successes have indicated that it is now essential to establish rapid, convenient and economical PCR-based assays in wheat breeding. In order to detect genes simultaneously in a single PCR, multiplex PCR can be developed, in which several markers in the same reaction mix are co-amplified under identical conditions. Two multiplex PCR assays, developed for the identification of genes/loci *ω-secalin*, *Glu-B1-2a*, *Glu-D1-1d*, *Glu-A3d*, *Glu-B3*, *Pin-D1b*, *Ppo-A1*, *Ppo-D1* and *Wx-B1b*, provide the proof-of-concept for the efficient screening of genotypes (Zhang et al. 2008a). A clear challenge is for multiplexing markers to have similar annealing temperatures for the different primers and for the expected PCR products to be easily separated on agarose gels (Ma et al. 2003; Wan et al. 2008).

Although six genes conferring disease resistance have been cloned in wheat, the FMs are available for only two genes (Sup-Table 3). If alleles conferring specific resistance are being sought, it is important to know which alleles are effective and potentially useful to local breeding programs. A good example is for the leaf rust resistance genes *Lr10* and *Lr21*, which confer resistance to a broad spectrum of *Puccinia triticina* races, but FMs are not available for these two genes because the reactions of alleles to various *Puccinia triticina* races have not been well characterized. Currently, FMs are being increasingly adopted in wheat breeding. Many FMs (Sup-Table 1) associated with wheat quality genes, in particular, are available; however, more FMs are needed for important traits such as disease and stress resistance in order to strengthen the application of molecular markers in breeding programs. SNPs are the most applicable markers for high-throughput screening once the genotype–phenotype associations are determined. The expanded use of these markers will develop as high-throughput techniques for MAS based on functional SNP markers and chips are established. The meaningful interpretation of whole genome studies to associate SNPs with variation in phenotype is expected to provide the next generation of FMs for use in wheat breeding.

Acknowledgments The authors are very grateful to Prof. Robert McIntosh, University of Sydney, Australia, for reviewing this manuscript. These works were supported by National Natural Science Foundation of China (31161140346 and 30830072).

References

Andersen JR, Lübberstedt T (2003) Functional markers in plant. Trends Plant Sci 8:554–560

Bagge M, Lübberstedt T (2008) Functional markers in wheat: technical and economic aspects. Mol Breed 22:319–328

Bagge M, Xia XC, Lübberstedt T (2007) Functional markers in wheat. Curr Opin Plant Biol 10:211–216

Beales J, Turner A, Griffiths S, Snape JW, Laurie DA (2007) A pseudo-response regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). Theor Appl Genet 115:721–733

Brevis JC, Dubcovsky J (2008) Effect of the *Gpc-B1* region from *Triticum turgidum* ssp. *dicoccoides* on grain yield, thousand grain weight and protein yield, pp 1–3. In: Appels R, Eastwood R, Lagudah E, Langridge P, Mackay M, McIntyre L, Sharp P (eds) Proceedings of 11th International Wheat Genetics Symposium, Brisbane Australia, 24–29 August 2008. Sydney University Press http://hdl.handle.net/2123/3179

Chai JF, Zhou RH, Jia JZ, Liu X (2006) Development and application of a new codominant PCR marker for detecting 1BL·1RS wheat–rye chromosome translocations. Plant Breed 125:302–304

Chen YH, Hunger RM, Carver BF, Zhang HL, Yan LL (2009) Genetic characterization of powdery mildew resistance in U.S. hard winter wheat. Mol Breed 24:141–152

Chen F, Zhang FY, Xia XC, Dong ZD, Cui DQ (2012) Distribution of puroindoline alleles in bread wheat cultivars of the Yellow and Huai valley of China and discovery of a novel puroindoline a allele without PINA protein. Mol Breed. doi:10.1007/s11032-011-9553-2

Chu CG, Tan CT, Yu GT, Zhong S, Xu SS, Yan LL (2011) A novel retrotransposon inserted in the dominant *Vrn-B1* allele confers spring growth habit in tetraploid wheat (*Triticum turgidum* L.). G3 (Genes, Genomes, Genetics) 1:637–645

D'Ovidio R, Anderson OD (1994) PCR analysis to distinguish between alleles of a member of a multigene family correlated with wheat bread-making quality. Theor Appl Genet 88:759–763

Devos KM (2005) Updating the 'crop circle'. Curr Opin Plant Biol 8:155–162

Distelfeld A, Fahima T (2007) Wild emmer wheat as a source for high-grain-protein genes: map-based cloning of *Gpc-B1*. Israel J Plant Sci 55:297–306

Distelfeld A, Uauy C, Fahima T, Dubcovsky J (2006) Physical map of the wheat high-grain protein content gene *Gpc-B1* and development of a high-throughput molecular marker. New Phytol 169:753–763

Dong CH, Ma ZY, Xia XC, Zhang LP, He ZH (2012) Allelic variation at the *TaZds-A1* locus on wheat chromosome 2A and development of a functional marker in common wheat. Agric Sci China (in press)

Ellis MH, Spielmeyer W, Gale KR, Rebetzke GJ, Richards RA (2002) "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. Theor Appl Genet 105:1038–1042

Fedorova L, Fedorov A (2003) Introns in gene evolution. Genetica 118:123–131

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100:15253–15258

Froidmont DD (1998) A co-dominant marker for the 1BL 1RS wheat rye translocation via multiplex PCR. J Cereal Sci 27:229–232

Fu DL, Szűcs P, Yan LL, Helguera M, Skinner JS, Zitzewitz JV, Hayes PM, Dubcovsky J (2005) Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol Gen Genomics 273:54–65

Fu DL, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen XM, Sela H, Fahima T, Dubcovsky J (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323:1357–1360

Gazza L, Nocente F, Ng PKW, Pogna NE (2005) Genetic and biochemical analysis of common wheat cultivars lacking puroindoline a. Theor Appl Genet 110:470–478

Geng HW, He ZH, Zhang LP, Qu YY, Xia XC (2012) Cloning the lipoxygenase gene on chromosome 4BS and development of functional markers in common wheat. Crop Sci 52. doi: 10.2135/cropsci2011.07.0365

Gennaro A, Koebner RMD, Ceoloni C (2009) A candidate for *Lr19*, an exotic gene conditioning leaf rust resistance in wheat. Funct Integr Genomics 9:325–334

Gill RW, Sanseau P (2000) Rapid in silico cloning of genes using expressed sequence tags (ESTs). Biotechnol Annu Rev 5:25–44

Giroux MJ, Morris CF (1997) A glycine to serine change in puroindoline b is associated with wheat grain hardness and low levels of starch-surface friabilin. Theor Appl Genet 95:857–864

Gupta PK, Rustgi S (2004) Molecular markers from the transcribed/expressed region of the genome in higher plants. Funct Integr Genomics 4:139–162

Gupta RB, Singh NK, Shepherd KW (1989) The cumulative effects of allelic variation in LMW and HMW glutenin subunits on physical dough properties in the progeny of two bread wheats. Theor Appl Genet 77:57–62

Gupta PK, Kumar J, Mir RR, Kumar A (2009) Marker-assisted selection as a component of conventional plant breeding. Plant Breed Rev 33:145–217

Gupta PK, Langridge P, Mir RR (2010) Marker-assisted wheat breeding: present status and future possibilities. Mol Breed 26:145–161

He ZH, Liu L, Xia XC, Liu JJ, Peña RJ (2005) Composition of HMW and LMW glutenin subunits and their effects on dough properties, pan bread, and noodle quality of Chinese bread wheats. Cereal Chem 82:345–350

He XY, He ZH, Zhang LP, Sun DJ, Morris CF, Fuerst EP, Xia XC (2007) Allelic variation of polyphenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat. Theor Appl Genet 115:47–58

He XY, Zhang YL, He ZH, Wu YP, Xiao YG, Ma CX, Xia XC (2008) Characterization of phytoene synthase 1 gene (*Psy1*) located on common wheat chromosome 7A and development of a functional marker. Theor Appl Genet 116:213–221

He XY, He ZH, Ma W, Appels R, Xia XC (2009a) Allelic variants of phytoene synthase 1 (*Psy1*) genes in Chinese and CIMMYT wheat cultivars and development of functional markers for flour colour. Mol Breed 23:553–563

He XY, Wang JW, Ammar K, Peña RJ, Xia XC, He ZH (2009b) Allelic variants at the *Psy-A1* and *Psy-B1* loci in durum wheat and their associations with grain yellowness. Crop Sci 49:2058–2064

Helguera M, Khan IA, Dubcovsky J (2000) Development of PCR markers for wheat leaf rust resistance gene *Lr47*. Theor Appl Genet 101:625–631

Helguera M, Khan IA, Kolmer J, Lijavetzky D, Liu ZQ, Dubcovsky J (2003) PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci 43:1839–1847

Helguera M, Vanzetti L, Soria M, Khan IA, Kolmer J, Dubcovsky J (2005) PCR markers for *Triticum speltoides* leaf rust resistance gene *Lr51* and their use to develop isogenic hard red spring wheat lines. Crop Sci 45:728–734

Howitt CA, Pogson BJ (2006) Carotenoid accumulation and function in seeds and non-green tissues. Plant Cell Environ 29:435–445

Huang J, Chen J, Yu W, Shyur L, Wang A, Sung H, Lee P, Su JC (1996) Complete structures of three rice sucrose synthase isogenes and differential regulation of their expressions. Biosci Biotechnol Biochem 60:233–239

Huang L, Brooks SA, Li WL, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655–664

Ishikawa G, Nakamura T (2007) A new co-dominant PCR-based marker to identify the high-molecular-weight glutenin subunit combination "5+10" of common wheat. Wheat Inf Serv 103:1–4

Jiang QY, Hou J, Hao CY, Wang LF, Ge HG, Dong YS, Zhang XY (2011) The wheat (*T. aestivum*) sucrose synthase 2 gene (*TaSus2*) active in endosperm development is associated with yield traits. Funct Integr Genomics 11:49–61

Jin H, Yan J, Peña RJ, Xia XC, Morgounov A, Han LM, Zhang Y, He ZH (2011) Molecular detection of high- and low-molecular-weight glutenin subunit genes in common wheat cultivars from 20 countries using allele-specific markers. Crop Pasture Sci 62:746–754

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Kruger JE, Matsuo RR, Preston K (1992) A comparison of methods for the prediction of Cantonese noodle colour. Can J Plant Sci 72:1021–1029

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theor Appl Genet 119:889–898

Laroche A, Frick MM, Huel R, Nykiforuk C, Conner B, Kuzyk A (2000) Molecular identification of the wheat stripe rust resistance gene *Yr10*, the first full-length leucine zipper-nucleotide binding site-leucine-rich-repeat resistance gene in cereals. http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=11990496

Lei ZS, Gale KR, He ZH, Gianibelli C, Larroque O, Xia XC, Butow BJ, Ma W (2006) Y-type gene specific markers for enhanced discrimination of high-molecular weight glutenin alleles at the *Glu-B1* locus in hexaploid wheat. J Cereal Sci 43:94–101

Liang D, Tang JW, Peña RJ, Singh R, He XY, Shen XY, Yao DN, Xia XC, He ZH (2010) Characterization of CIMMYT bread wheats for high- and low-molecular weight glutenin subunits and other quality-related genes with SDS-PAGE, RP-HPLC and molecular markers. Euphytica 172:235–250

Lillemo M, Skinnes H, Brown JKM (2010) Race specific resistance to powdery mildew in Scandinavian wheat cultivars, breeding lines and introduced genotypes with partial resistance. Plant Breed 129:297–303

Liu C, Yang ZJ, Li GR, Zeng ZX, Zhang Y, Zhou JP, Liu ZH, Ren ZL (2008a) Isolation of a new repetitive DNA sequence from *Secale africanum* enables targeting of Secale chromatin in wheat background. Euphytica 159:249–258

Liu SX, Chao SM, Anderson JA (2008b) New DNA markers for high molecular weight glutenin subunits in wheat. Theor Appl Genet 118:177–183

Liu L, Ikeda TM, Branlard G, Peña R, Rogers WJ, Lerner SE, Kolman MA, Xia XC, Wang LH, Ma W, Appels R, Yoshida H, Wang AL, Yan YM, He ZH (2010) Comparison of low molecular weight glutenin subunits identified by SDS-PAGE, 2-DE, MALDI-TOF-MS and PCR in common wheat. BMC Plant Biol 10:124

Lübberstedt T, Zein I, Andersen JR, Wenzel G, Krützfeldt B, Eder J, Ouzunova M, Chun S (2005) Development and application of functional markers in maize. Euphytica 146:101–108

Luo C, Griffin WB, Branlard G, McNeil DL (2001) Comparison of low- and high- molecular-weight wheat glutenin allele effects on flour quality. Theor Appl Genet 102:1088–1098

Ma W, Zhang W, Gale KR (2003) Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica 134:51–60

Ma DY, Yan J, He ZH, Wu L, Xia XC (2012) Characterization of a cell wall invertase gene *TaCwi-A1* on common wheat chromosome 2A and development of functional markers. Mol Breed 29:43–52

Matsumoto T, Wu JZ, Kanamori H, Katayose Y, Fujisawa M et al (2005) The map-based sequence of the rice genome. Nature 436:793–800

Miskelly DM (1984) Flour components affecting paste and noodle colour. J Sci Food Agric 35:463–471

Mohler V, Bauer A, Bauer C, Flath K, Schweizer G, Hartl L (2011) Genetic analysis of powdery mildew resistance in German winter wheat cultivar Cortez. Plant Breed 130:35–40

Nakamura T, Vrinten P, Saito M, Konda M (2002) Rapid classification of partial waxy wheat using PCR-based markers. Genome 45:1150–1156

Parker GD, Chalmers KJ, Rathgen AJ, Langridge P (1998) Mapping loci associated with flour colour in wheat (*Triticum aestivum* L.). Theor Appl Genet 97:238–245

Peusha H, Enno T, Jakobson I, Tsõmbalova J, Ingver A, Järve K (2008) Powdery mildew resistance of Nordic spring wheat cultivars grown in Estonia. Acta Agric Scand Sect B-Soil Plant Sci 58:289–296

Ragupathy R, Naeem HA, Reimer E, Lukow OM, Sapirstein HD, Cloutier S (2008) Evolutionary origin of the segmental duplication encompassing the wheat *GLU-B1* locus encoding the overexpressed Bx7 ($Bx7^{OE}$) high molecular weight glutenin subunit. Theor Appl Genet 116:283–296

Ram S, Sharma S, Verma A, Tyagi BS, Peña RJ (2011) Comparative analyses of LMW glutenin alleles in bread wheat using allele-specific PCR and SDS-PAGE. J Cereal Sci 54:488–493

Ravel C, Praud S, Murigneux A, Canaguier A, Sapet F, Samson D, Balfourier F, Dufour P, Chalhoub B, Brunel D, Beckert M, Charmet G (2006) Single-nucleotide polymorphism frequency in a set of selected lines of bread wheat (*Triticum aestivum* L.). Genome 49:1131–1139

Reddy ASN (2007) Alternative splicing of pre-messenger RNAs in plants in the genomic era. Annu Rev Plant Biol 58:267–294

Saito M, Vrinten P, Ishikawa G, Graybosch R, Nakamura T (2009) A novel codominant marker for selection of the null *Wx-B1* allele in wheat breeding programs. Mol Breed 23:209–217

Schnable PS, Ware D, Fulton RS, Stein JC, Wei FS et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115

Schwarz G, Felsenstein FG, Wenzel G (2004) Development and validation of a PCR-based marker assay for negative selection of the HMW glutenin allele *Glu-B1-1d* (*Bx-6*) in wheat. Theor Appl Genet 109:1064–1069

Singh R, Goutam U, Gupta RK, Pandey GC, Shoran J, Tiwari R (2009) Allelic variations of functional markers for polyphenol oxidase (PPO) genes in Indian bread wheat (*Triticum aestivum* L.) cultivars. J Genet 88:325–329

Smith RL, Schweder ME, Barnett RD (1994) Identification of glutenin alleles in wheat and triticale using PCR-generated DNA markers. Crop Sci 34:1373–1378

Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39:623–630

Su ZQ, Hao CY, Wang LF, Dong YC, Zhang XY (2011) Identification and development of a functional marker of *TaGW2* associated with grain weight in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 122:211–223

Sun DJ, He ZH, Xia XC, Zhang LP, Morris CF, Appels R, Ma WJ, Wang H (2005) A novel STS marker for polyphenol oxidase activity in bread wheat. Mol Breed 16:209–218

Sun YW, He ZH, Ma WJ, Xia XC (2011) Alternative splicing in the coding region of *Ppo-A1* directly influences the polyphenol oxidase activity in common wheat (*Triticum aestivum* L.). Funct Integr Genomics 11:85–93

Talbert LE, Blake NK, Chee PW, Blake TK, Magyar GM (1994) Evaluation of "sequence-tagged-site" PCR products as molecular markers in wheat. Theor Appl Genet 87:789–794

Tommasini L, Yahiaoui N, Srichumpa P, Keller B (2006) Development of functional markers specific for seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. Theor Appl Genet 114:165–175

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298–1301

Varshney RK, Graner A, Sorrells ME (2005) Genomics-assisted breeding for crop improvement. Trends Plant Sci 10:621–630

Varshney RK, Beier U, Khlestkina EK, Kota R, Korzun V, Graner A, Börner A (2007) Single nucleotide polymorphisms in rye (*Secale cereale* L.): discovery, frequency, and applications for genome mapping and diversity studies. Theor Appl Genet 114:1105–1116

Vogel JP, Garvin DF, Mockler TC et al (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763–768

Wan YX, Zhang XK, Xia XC, Zhang PZ, He ZH (2008) Development of multiplex PCR and identification of major quality genes in cultivars from Yellow and Huai River Valley Wheat Region. Sci Agric Sin 41:643–653

Wang BB, Brendel V (2006) Genome wide comparative analysis of alternative splicing in plants. Proc Natl Acad Sci 103:7175–7180

Wang E, Wang JJ, Zhu XD, Wei H, Wang LY, Li Q, Zhang LX, He W, Lu BR, Lin HX, Ma H, Zhang GQ, He ZH (2008) Control of rice grain-filling and yield by a gene with a potential signature of domestication. Nat Genet 40:1370–1374

Wang JW, He XY, He ZH, Wang H, Xia XC (2009a) Cloning and phylogenetic analysis of phytoene synthase 1 (*Psy1*) genes in common wheat and related species. Hereditas 146:208–256

Wang LH, Zhao XL, He ZH, Ma W, Appels R, Peña RJ, Xia XC (2009b) Characterization of low-molecular-weight glutenin subunit *Glu-B3* genes and development of STS markers in common wheat (*Triticum aestivum* L.). Theor Appl Genet 118:525–539

Wang LH, Li GY, Peña RJ, Xia XC, He ZH (2010) Development of STS markers and establishment of multiplex PCR for *Glu-A3* alleles in common wheat (*Triticum aestivum* L.). J Cereal Sci 51:305–312

Wei B, Jing RL, Wang CS, Chen JB, Mao XG, Chang XP, Jia JZ (2009) *Dreb1* genes in wheat (*Triticum aestivum* L.):

development of functional markers and gene mapping based on SNPs. Mol Breed 23:13–22

Wu L, Xia XC, Zhu HZ, Li SZ, Zheng YL, He ZH (2010) Molecular characterization of *Lr34/Yr18/Pm38* in 273 CIMMYT wheat cultivars and lines using functional markers. Sci Agric Sin 43:4553–4561

Xiao YG, He XY, Liu JJ, Sun DJ, Xia XC, He ZH (2008) Molecular identification and distribution of the polyphenol oxidase genes in Chinese winter wheat cultivars. Sci Agric Sin 41:954–960

Yahiaoui N, Srichumpa P, Dudler R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J 37:528–538

Yamamori M, Quynh NT (2000) Differential effects of Wx-A1, -B1 and –D1 protein deficiencies on apparent amylose content and starch pasting properties in common wheat. Theor Appl Genet 100:32–38

Yan LL, Helguera M, Kato K, Fukuyama S, Sherman J, Dubcovsky J (2004a) Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theor Appl Genet 109:1677–1686

Yan LL, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004b) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1643

Yan LL, Fu DL, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc Natl Acad Sci USA 103:19581–19586

Yang FP, He XY, He ZH, Shang XW, Yang WX, Xia XC (2008) Molecular detection and distribution of allelic variations of a gene for yellow pigment content in Chinese winter wheat cultivars. Sci Agric Sin 41:2923–2930

Yang FP, Zhang XK, Xia XC, Laurie DA, Yang WX, He ZH (2009) Distribution of the photoperiod insensitive *Ppd-D1a* allele in Chinese wheat cultivars. Euphytica 165:445–452

Zhang W, Gianibelli MC, Rampling LR, Gale KR (2004) Characterisation and marker development for low molecular weight glutenin genes from *Glu-A3* alleles of bread wheat (*Triticum aestivum* L). Theor Appl Genet 108:1409–1419

Zhang XK, Yang SJ, Zhou Y, He ZH, Xia XC (2006) Distribution of the *Rht-B1b*, *Rht-D1b* and *Rht8* reduced height genes in autumn-sown Chinese wheats detected by molecular markers. Euphytica 152:109–116

Zhang XK, Liu L, He ZH, Sun DJ, He XY, Xu ZH, Zhang PP, Chen F, Xia XC (2008a) Development of two multiplex PCR assays targeting improvement of bread-making and noodle qualities in common wheat. Plant Breed 127:109–115

Zhang XK, Xiao YG, Zhang Y, Xia XC, Dubcovsky J, He ZH (2008b) Allelic variation at the vernalization genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3* in Chinese wheat cultivars and their association with growth habit. Crop Sci 48:458–470

Zhang CY, Dong CH, He XY, Zhang LP, Xia XC, He ZH (2011) Allelic variation at the *TaZds-D1* locus on wheat chromosome 2DL and their association with yellow pigment content. Crop Sci 51:1580–1590