Molecular Marker Development and Application for Improving Qualities in Bread Wheat

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Abstract Molecular marker technology has provided a novel and efficient tool for improving qualities in bread wheat. This chapter summarizes progress in gene cloning, gene specific marker (functional marker) development and validation, establishment of high-throughput platform in genotyping, as well as integration of molecular marker technology with conventional quality testing and traditional breeding since 2000. Comparative genomic approach was used to discover more than 20 loci controlling important quality traits, and to develop and validate around 66 gene-specific markers for quality traits such as high- and low-molecular-weight glutenin subunits, color associated traits including polyphenol oxidase (PPO) and yellow pigment, as well as starch parameters. Now the availability of reference wheat genome sequence and on-going efforts to sequence diverse wheat cultivars would offer new opportunities to identify loci responsible for various quality traits through improved genome-wide association study (GWAS) and analytical approaches. Development of high-throughput genotyping platform such as SNP arrays, genotyping-by-sequencing (GBS) and Kompetitive Allele-specific PCR (KASP) have been well-established and will accelerate molecular breeding progress for quality improvement. New cultivars carrying excellent bread-making quality and outstanding agronomic performance such as Zhongmai 1062 and Jimai 23 were developed. Future strategies in using molecular markers in the context of gene-editing to fine tune allelic effects are also discussed.

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1 Methodology for Molecular Marker Development and Validation

The research area spanning wheat glutenins during the last 30 years is a classic example of evolution in diagnostic platforms used in wheat end-use quality improvement. The advances in molecular genetics and biochemistry have provided the basis for understanding the genetics, structure and composition of glutenins in wheat. The genes controlling HMW-GS are mapped to *Glu-1* loci on the long arms of homoeologous group 1 chromosomes named as *Glu-A1*, *Glu-B1* and *Glu-D1* (Payne 1987). SDS-PAGE was considered to be the simplest and commonly used technique to identify HMW-GS and LMW-GS alleles. Advances in molecular biology have overcome the low-resolution limitations of protein-based identification of HMW glutenin allele by application of specific PCR markers.

Development of these markers is based on DNA polymorphisms among the glutenin subunit genes and once available they can be considered as perfect or functional markers for HMW-GS alleles. The major advantages are high-throughput capability and genotyping at the vegetative stages (Liu et al. 2008). A total of 12 allele-specific markers at *Glu-1* loci have been reported, however only eight of them are frequently used in breeding (Table 1). Allele-specific PCR (AS-PCR) markers are available for the three most common x-type subunits at the *Glu-A1* locus i.e. *1Ax2∗, 1Ax1* and *1Ax Null* (Liu et al. 2008; Ma et al. 2003) (Table 1). At *Glu-B1*, allele-specific markers are available for x-type subunits *Bx7*, *Bx14*, and *Bx17* (Xu et al. 2008), *Bx6* (Schwarz et al. 2004), $Bx7^{OE}$ (Butow et al. 2003; Ragupathy et al. 2008), and y-type subunits *By8*, *By16* and *By18* (Lei et al. 2006). At *Glu-D1*, markers are available to identify *1Dx2*, *1Dx5*, *1Dy10* and *1Dy12* (Liu et al. 2008). Out of six *Glu-1* genes, the gene *Glu-A1y* is usually not expressed in bread or durum wheat. Roy et al. (2018) introduced 1Ay gene in two Australian bread wheat cultivars, Livingston and Bonnie Rock, and three sister lines were developed. The introduction of 1Ay gene increased glutenin/gliadin ratio without affecting the relative amount of subunits, and increased gluten contents by 10%.

However, there is limited expertise in the world to diagnose LMW-GS alleles in bread and durum wheat. SDS-PAGE, 2-DE, MALDI-TOF-MS and PCR based markers were developed to detect the *Glu-3* allelic variation. Liu et al. (2010) compared the four techniques to assess their suitability for use in breeding programs. They indicated that PCR-based markers are the simplest, most accurate, lowest cost technique and therefore recommended them for the identification of *Glu-A3* and *Glu-B3* alleles in breeding programs. Seventeen allele-specific markers for *Glu-A3* and *Glu-B3* loci have been reported and used (Table 1), and multiplex PCR protocols have been developed to reduce costs of screening in practical breeding programs (Wang et al. 2010). Application of functional markers for identification of LMW-GS in various types of wheat germplasm has also been reported (Jin et al. 2011). Recently, the allelic differentiation of *Glu-3* loci was further differentiated using haplotype analysis and some more diagnostic markers were developed to identify *Glu-B3c* and *Glu-B3d* alleles (Ibba et al. 2017) (Table 1).

Table 1 List of all functional markers available in wheat along with their KASP counterpart and standard cultivars for allele identification

Trait	Gene	Marker	Allele	KASP[a]	Standard	Reference
Gluten elasticity	*Glu-A1*	UMN19	*Glu-A1* (Ax1, Ax2[a], AxNull)	gluA1.1_1594; gluA1.1_1883	Chinese Spring (CS), Opata 85,	Liu et al. 2008
	Glu-A1	Ax2[a]	*Glu-A1b* (Ax2[a])	As above	Pavon 76, Opata 85	Ma et al. 2003
	Glu-B1	TaBAC1215C06-F517/ R964	*Glu-B1al* ($Bx7^{OE}$)	$Bx7^{OE}$	Dorico, ProINTA Colibr 1, Klein Jabal	Ragupathy et al. 2008
	Glu-B1	cauBx642	*Glu-B1b* (7 + 8); *Glu-B1i* (17 + 18); *Glu-B1h* (14 + 15)	NA	CS, Jing771, Pm97034	Xu et al. 2008
	Glu-B1	ZSBy9F2/R2	*Glu-B1f* (13 + 16)	NA	Baxter	Lei et al. 2006
	Glu-B1	ZSBy8F5/By8R5	*Glu-B1* (By8)	NA	Sunco	Lei et al. 2006
	Glu-D1	UMN25F/25R	*Glu-D1* (Dx2, Dx5)	Glu-D1d_SNP	CS, Pavon 76	Liu et al. 2008
	Glu-D1	UMN26F/26R	*Glu-D1* (Dy10, Dy12)	Glu-D1d_SNP	CS, Pavon 76	Liu et al. 2008
	Glu-A3	LA1F/SA1R	*Glu-A3a*	NA	Neixiang 188, Chinese Spring	Wang et al. 2010
	Glu-A3	LA3F/SA2R	*Glu-A3b*	NA	Gabo, Pavon 76	Wang et al. 2010
	Glu-A3	LA1F/SA3R	*Glu-A3c*	NA	Pitic, Seri 82	Wang et al. 2010
	Glu-A3	LA3F/SA4R	*Glu-A3d*	NA	Nidera Baguette 10, Cappelle-Desprez	Wang et al. 2010
	Glu-A3	LA1F/SA5R	*Glu-A3e*	NA	Amadina, Marquis	Wang et al. 2010
	Glu-A3	LA1F/SA6R	*Glu-A3f*	NA	Kitanokaori, Renan	Wang et al. 2010
	Glu-A3	LA1F/SA7R	*Glu-Ag*	NA	Bluesky, Glenlea	Wang et al. 2010

(continued)

Table 1 (continued)

Trait	Gene	Marker	Allele	KASP[a]	Standard	Reference
	Glu-B3	SB1F/SB1R	*Glu-B3a*	NA	Chinese Spring	Wang et al. 2009
	Glu-B3	SB2F/SB2R	*Glu-B3b*	NA	Renan, Gabo	Wang et al. 2009
	Glu-B3	SB3F/SB4R	*Glu-B3c*	NA	Insignia, Halberd	Wang et al. 2009
	Glu-B3	SB4F/SB4R	*Glu-B3d*	NA	Pepital, Ernest	Wang et al. 2009
	Glu-B3	SB5F/SB5R	*Glu-B3e*	NA	Cheyenne	Wang et al. 2009
	Glu-B3	SB6F/SB6R	*Glu-B3fg*	NA	Fengmai 27	Wang et al. 2009
	Glu-B3	SB7F/SB7R	*Glu-B3g*	NA	Splendor, Cappelle-Desprez	Wang et al. 2009
	Glu-B3	SB8F/SB8R	*Glu-B3h*	NA	Aca 303, Pavon 76	Wang et al. 2009
	Glu-B3	SB9F/SB9R	*Glu-B3ad*	NA	Opata 85	Wang et al. 2009 Ikeda unpublished
	Glu-B3	SB10F/SB10R	*Glu-B3bef*	NA	Gawain	Wang et al. 2009
Grain texture	*Pina-D1*	Pina-N2	*Pina-D1a,b*	Pina-D1_INS	Chinese Spring, Zhongyou 9507	Chen et al. 2012
	Pinb-D1	Pinb-D1	*Pinb-D1a,b*	Pinb-D1_INS	Chinese Spring, Lorvin 10	Giroux and Morris 1997
	Pinb-D1	Pinb-DF/Pinb-DR	*Pinb-D1p*	No	Shannongyoumai 3	Li et al. 2008

	Pinb-B2	Pinb-B2v2	*Pinb-B2a, b*	Pinb2_IND	Chinese Spring, Zhongmai 175	Chen et al. 2010
Polyphenol oxidase	*Ppo-A1*	PPO18 and PPO33	*Ppo-A1a,b*	PPOA1_SNP	Zhengmai 9023, Jinmai 67	Sun et al.2005
	Ppo-D1	PPO16 and PPO29	*Ppo-D1a,b*	PPOD1_SNP	Chinese Spring, Mexipak-65	He et al.2007
Lipoxygenase	*TaLox-B1*	LOX16	*TaLox-B1a*	LoxB1_SNP	Chinese Spring	Geng et al. 2012
	TaLox-B1	LOX18	*TaLox-B1b*	LoxB1_SNP	Inqilab-91	Geng et al. 2012
	TaLox-B2	LOX-B23	*TaLox-B2a,b*	NA	Zhongmai 18, GC8901	Zhang et al. 2015
Phytoene synthase	*Psy-A1*	YP7A	*Psy-A1a,b*	PSY-A1_IND	Nongda 3291, Wanmai 33	He et al.2008
	Psy-B1	YP7B-1	*Psy-B1a,b*	NA	Jingdong 8, Jimai 38	He et al.2009
	Psy-B1	YP7B-2	*Psy-B1c*	PSY_B1c_SNP	Yannong 18	He et al. 2009
	Psy-D1	YP7D-1	*Psy-D1a,g*	Psy1Da-g	Chinese Spring, Zhou 8425B	Wang et al. 2009
Zeta-carotene desaturase	*TaZds-A1*	YP2A-1	*TaZds-A1a,b*	ZDS-A1_SNP	Chinese Spring, Zhongmai 175	Dong et al. 2012
	TaZds-D1	YP2D-1	*TaZds-D1a,b*	ZDS-D1_SNP	Chinese Spring, Sunstate	Zhang et al. 2011
Lycopene	*Lyce-B1*	NA	*TaLYC-B1a,b*	LYCE-B1_SNP	Zhoumai 8235B, Norin 61	Dong unpublihed
Phytoene desaturase	*PDS-B1*	NA	*TaPds-B1a,b*	PDS-B1_SNP	Zhou 8425B, Insignia	Dong unpublished
Peroxidase	*POD-A1*	POD-3A1,2	*TaPod-A1a,b*	PODA1_462_SNP	Norin 61, Norin 67	Wei et al.2015

(continued)

Table 1 (continued)

Trait	Gene	Marker	Allele	KASP[a]	Standard	Reference
Avenin-like protein	*ALPb-7A*	NA	*NA*	ALPb7A-3IND	Chinese Spring, Chara, Westonia	Chen et al. 2016
	ALPb-7A	NA	*NA*	ALPb7A_225SNP	Chinese Spring, Chara, Westonia	Chen et al. 2016
	ALPb-4A	TaALP-7A-F/R	*active-type ALP-7A*	ALPb4A_228_SNP	Chinese Spring, Chara, Westonia	Chen et al. 2016
	ALPb-4A	NA	*NA*	ALPb4A_773_SNP	Chinese Spring, Yitip	Chen et al. 2016
	ALPb-4A	NA	*NA*	ALPb4A_3IND	Chinese Spring, Chara, Westonia, Yitip	Chen et al. 2016
	ALPa-4A	NA	CS-type, Spitfire-type	ALPa4A_285_SNP	Spitfire	Chen et al. 2016
	ALPa-4A	NA	Wyalketchem-type	ALPa4A_184_SNP	Wyalketchem	Chen et al. 2016
Pre-harvest sprouting	*TaSdr-A1*	Sdr2A	*TaSdr-A1a,b*	SDRA1_643	Yangxiaomai, Zhongyou 9507	Zhang et al. 2014
	TaSdr-B1	Sdr2B	*TaSdr-B1a,b*	SDR_SNP	Yangxiaomai/Zhongyou 9507	Zhang et al., 2014
	Vp1-B1	Vp1B3	*Vp1-B1a,c*	Vp1B1-83_IND	Zhongyou 9507, Xinong 979	Yang et al. 2007
	Vp1-B1	Vp1B3	*Vp1-B1a,b*	Vp1B1-193_IND	Zhongyou 9507, Yongchuanbaimai	Yang et al. 2007
	Vp-1A	A17–19	*Vp-1Ab, c*	NA	Wanxianbaimaizi, Jing411	Chang et al. 2011
	TaDFR	DFR-F/R	TaDFR-Ba, b	NA	Taiyuan 566, Longmai 13	Bi et al. 2014
	Phs1	TaPHS1-SNP1	*Rio-type, NW-type*		RioBlanceo, NW97S186	Liu et al. 2013

Grain color	*Tamyb10-A1*	Tamyb10-A1	*R-A1a,b*	Tamby10-A1	Norin 10, Norin 61, Chinese Spring; Prina	Himi et al. 2011
	Tamyb10-A1	Tamyb10-A1	*R-A1a,* Norin-type	Tamby10-Nor17	Norin 10, Norin 61, Chinese Spring	Himi et al. 2011
	Tamyb10-B1	Tamyb10-B1	*R-B1a,b*	TamybR B1a-b	Norin 10, Norin 61,Chinese Spring	Himi et al. 2011
	Tamyb10-D1	Tamyb10-D1	*R-D1a,b*	TamybR D1a-b	Norin 10, Norin 61, Chinese Spring	Himi et al. 2011
	Tamyb10-D1	Tamyb10D	*Not designated*	NA	Yangxiaomai, Zhongyou 9507	Wang et al. 2016
Amylose content	*Wx-A1*	AFC/AR2	Null, Wild-type	NA	Norin 61, Kanton 107	Nakamura et al. 2002
	Wx-B1	BDFL/BRD	Null, Wild-type	WxB1_SNP	Norin 61, Kanton 107	Nakamura et al. 2002
	Wx-D1	BDFL/DRSL	Null, Wild-type	NA	Norin 61, California	Nakamura et al. 2002
Wheat bread-making quality	Wbm	NWPFor/Rev		Wbm_SNP	Mantol, Aca 601, Insignia	Furtado et al. 2015

[a]KASP markers are partially reported in Rasheed et al. (2016). Further information on other KASP markers can be obtained by personal communication to Zhonghu He or Awais Rasheed

A combination of different techniques was required to identify certain alleles of LMW-GS and these combinations are especially useful when characterizing new alleles. As more alleles are reported at *Glu-A3* and *Glu-B3* in bread wheat, more molecular markers will be needed to distinguish them in breeding germplasm. Liu et al. (2010) recommended a standard set of 30 cultivars to represent all known LMW-GS allelic variants for future studies. Among them, Chinese Spring, Opata 85, Seri 82 and Pavon 76 were recommended as a core set for use in SDS-PAGE gels. Use of the standard cultivar set was recommended to promote and facilitate information sharing on LMW-GS in order to ultimately enhance the global quality improvement efficiency in wheat.

Functional markers are developed from the polymorphisms within the coding sequences of functional genes which could be either single nucleotide polymorphisms (SNPs) or InDels. Fine mapping followed by map-based cloning is the most effective strategy to isolate the functional genes in plants (Yan et al. 2004). However, due to a large genome size, it had been very difficult to clone genes by map-based cloning in bread wheat. Alternatively, a significantly large number of genes, especially several genes related to wheat quality, have been cloned in wheat using comparative genomics approach. There is very high gene collinearity (synteny) among the grass genomes of maize, barley, rice, and *Brachypodium* which could facilitate gene discovery in wheat (El Baidouri et al. 2017; Valluru et al. 2014). A classic example is the cDNA sequence of maize *Psy1* gene (GenBank accession U32636) in that 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 a functional marker YP7A for discrimination of two alleles at *Psy-A1* locus was developed and validated using 217 Chinese cultivars and 240 $F_{2:6}$ lines from the cross of PH82–2/Neixiang 188. However, the recent reports of genome sequences of wheat and its immediate progenitors could facilitate the unprecedented discovery of functional genes and development of functional markers for use in wheat breeding (Rasheed et al. 2018).

Liu et al. (2012) documented 97 functional markers for detecting 93 alleles at 30 loci in bread wheat. This number has increased during the past 5 years due to rapid advancements in wheat genomics. Currently, there are 157 functional markers documented for more than 100 loci underpinning adaptability, grain yield, disease resistance, end-use quality and tolerance to abiotic stresses. Out of all these functional markers, almost 66 are related to end-use quality in wheat (Table 1).

2 Overview of Functional Markers Related to End-Use Quality in Wheat

While next generation sequencing (NGS) and SNP arrays are excellent choices for gene discovery and mapping, and for identifying linked markers for important traits. Such trait-associated markers, in addition to functional markers, are ideal for gene tagging and gene introgression in breeding. Functional markers for wheat end-use

quality have been described along with tester germplasm for identification of alleles. Apart from gene-specific markers for *Glu-1* and *Glu-3* loci, there are several other newly identified genes underpinning bread-making quality in wheat (Table 1). Recently, a highly expressed bread-making gene (*wbm*) was identified in the transcriptome of developing wheat seed (Furtado et al. 2015). RNA-seq analysis revealed that the S-rich *wbm* gene was highly expressed consistently in all cultivars with good bread-making quality. Guzmán et al. (2016) later identified 8 of 56 CIMMYT cultivars carrying the *wbm* gene and concluded that the allele has a significant effect on overall gluten quality, gluten strength, gluten extensibility and bread-making quality. However, the effects were smaller than those associated with the *Glu-B1* and *Glu-D1* loci. Similarly, the wheat avenin-like protein (ALP) is important constituent of gluten and has shown positive effects on dough properties. Chen et al. (2016) isolated wheat ALP genes and developed a functional marker to identity active and silenced b-type ALP-7A gene, where the active type had significant effect on bread-making quality.

Polyphenol oxidase (PPO) activity responsible for brown discoloration of the wheat products especially Asian noodles, is an undesirable character. Several markers have been developed to identify PPO alleles on chromosomes 2A and 2D (He et al. 2007; Sun et al. 2005). The practical usage of these markers in wheat breeding for identification of genotypes with lower PPO activity is scientifically valid (Liang et al. 2010). Nevertheless, PPO gene located on chromosome 2B had limited polymorphism in Chinese wheat to develop a functional marker. Lipoxygenase activity is also a major determinant of color and processing quality of wheat products (Geng et al. 2012). A lipoxygenase (LOX) gene has been mapped to chromosome 4BS (*TaLox-B1*) and two allele-specific markers LOX16 and LOX18 amplify 489- and 791- bp PCR fragments in cultivars with higher and lower LOX activities, respectively (Geng et al. 2012). The gene, *TaLox-B1*, was sequenced and a SNP was identified in the third exon which helped in development of two markers for identifying alleles *TaLox-B1a* and *TaLox-B1b.* Zhang et al.(2015) reported two new loci for *TaLox* on chromosome 4BS and are designated as *TaLox-B2* and *TaLox-B3*. They also developed a functional marker, Lox-B23, to distinguish *TaLox-B2a, TaLox-B2b* and *TaLox-B3* alleles in bread wheat.

The color of wheat derived products is due to the yellow pigment content (YPC). Regional preference for color does exist, like 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 (Parker et al. 1998). Carotenoids are responsible for yellow pigment (He et al. 2008) while phytoene synthase (PSY) and zeta-carotene desaturase (ZDS) are important enzymes in the biosynthetic pathway for carotenoid synthesis in wheat (Dong et al. 2012; Zhang et al. 2011). PSY genes are present on chromosomes 7AL, 7BL and 7DL and several allele-specific markers for PSY genes have been developed (He et al. 2008; He et al. 2009, Wang et al. 2009). The reverse genetics approaches using RNAi decreased the *Psy1* transcripts level by 54–76% and YPC was reduced by 26–35%. This indicated that PSY1 is the most important regulatory enzyme in carotenoid biosynthesis and a series of candidate genes involved in secondary metabolic pathways and core meta-

bolic processes responded to *Psy1* down-regulation (Zhai et al. 2016). Similarly, markers for ZDS genes on chromosomes 2A and 2D can discriminate allelic difference in wheat (Dong et al. 2012; Zhang et al. 2011). More recently, a novel QTL for peroxidase (POD) activity was mapped and was annotated to be *TaPod-A1* gene determining flour color (Wei et al. 2015). Two functional markers were developed for two alleles amplifying 291- and 766-bp fragments in cultivars with lower and higher POD activities, respectively. Nigro et al. (2017) identified six candidate genes involved in the biosythesis of hydroxycinnamic acid in wheat.

Starch fractions account for almost 70% of the dry matter in wheat grain and greatly affect end-use quality especially Asian noodles. Waxy proteins are the products of granule bound starch synthase (GBSS I) genes on chromosomes 7A, 4A and 7D of wheat. Nakamura et al. (Nakamura et al. 2002) developed functional markers for waxy- and wild-type alleles and validated the alleles in a set of 30 lines using a single PCR reaction. Later, a high-throughput KASP marker is also developed for *Wx-B1* locus and further development of KASP markers for other *Wx* loci is in progress. A waxy mutant line carrying *Wx-D1d* allele has been identified and characterized at molecular level (Yi et al. 2017) and a KASP marker was developed for *Wx-D1d* allele which was tracked in backcross derived populations.

The tolerance to pre-harvest sprouting (PHS) is an important breeding objective in many countries, however the work on gene discovery is very limited since reliable phenotyping is a time-consuming activity. Liu et al. (2013) cloned a major QTL related to PHS and designed the KASP marker for *Phs1* allele. Another major gene, *Viviparous 1* as a regulator of late embryo development have shown significant effect on sprouting tolerance, was cloned and functional markers were developed for three different alleles (Yang et al. 2007). Chang et al. (2011) identified six alleles at *Vp-1A* locus, however no allelic variation was found at *Vp-1D* locus. Similarly, functional markers are also available for seed dormancy genes, *TaSdr-B1* and *TaSdr-A1* (Zhang et al. 2017) genes, which are major factors in tolerance to PHS. Red-grained wheat varieties are generally more tolerant to PHS as compared to white-grained varieties. The red pigment of grain coat is synthesized through the flavonoid biosynthesis pathway, in which the dihydroflavonol-4-reductase gene (DFR) is one of the gene involved in anthocyanin synthesis. Bi et al. (2014) cloned homeologous genes *TaDFR* in Chinese wheat, and no allelic variation was found at *TaDFR-A1* and *TaDFR-D1* genes on chromosomes 3A and 3D. However, two alleles were identified at *TaDFR-B1* locus characterized by 8-bp InDel. A CAPS marker was developed to differentiate red and white grain Chinese cultivars with distinct PHS resistance. Similarly, major grain color gene *Tamyb10* (transcription factor for *R-1* gene) on chromosomes 3A, 3B and 3D have been cloned and functional markers are available to identify the allelic variations (Himi et al. 2011). Wang et al. (2016) further developed a new STS marker to characterize *Tamyb10-D* gene in Chinese wheat cultivars differing in response to PHS. They concluded that wheat cultivars with 1629-bp fragment for *Tamyb10-D* were tolerant to PHS as compared to cultivras amplifying 1178- bp fragment. Rasheed et al. (2016) converted several of these markers including *TaSdr, TaVp1-B1* and *TaMFT-A1* genes into high-throughput KASP assays. All these func-

tional markers for bread-making and processing quality provide a powerful toolkit to complement the phenotypic selection of wheat germplasm with desirable end-use quality features during breeding (Torada et al. 2016).

3 High-Throughput Genotyping for Wheat End-Use Quality

Almost all functional markers in wheat were gel-based markers, thus hinder the large-scale germplasm screening. Therefore, it is challenging to develop a high-throughput platform to use single markers in wheat breeding programs. Rasheed et al. (2017) highlighted six factors in developing such platforms; these included (i) number of data points that can be generated in a short time period, (ii) ease of use, (iii) data quality (sensitivity, reliability, reproducibility, and accuracy), (iv) flexibility (genotyping few samples with many SNPs or many samples with few SNPs), (v) assay development requirements, and (vi) genotyping cost per sample or data point. Sufficient recent reports indicate that LGC's KASP is an well-received global benchmark technology for such genotyping requirements in terms of both cost-effectiveness and high throughput (Semagn et al. 2014).

At a first step, several groups worked on converting gel-based functional markers to high-throughput KASP markers (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/kasp_download.php?URL=). The numbers were increased to 72 after validation in a bread wheat diversity panel (Rasheed et al. 2016). This effort has continued in our group and we currently have more than 150 KASP markers for almost 100 functional genes (Rasheed et al. unpublished data). Currently, most gene mapping studies (both QTL and GWAS) use SNP arrays or NGS; the markers linked to QTL are SNPs and can be easily converted to KASP assays for further diagnosis or QTL introgression in breeding. Similarly, diagnostic KASP assay development is preferred due to the wide acceptance and usefulness of this technology during functional gene discovery. Several QTL linked to quality traits and SNPs in functional genes have been converted to KASP markers; examples include *Glu-1, Glu-3, Pin-D1* and *Ppo-A1* (Rasheed et al. 2016) . The functional genes for which KASP markers are available are listed in Table 1.

KASP provides the throughput required in breeding programs for gene tagging and gene introgression without compromising of flexibility. However, higher cost is still an issue because KASP mastermix is a commercial proprietary from LGC and there are no other competitors. Due to this limitation, several groups tried to develop other open source uni-plex SNP genotyping techniques like semi-thermal asymmetric reverse PCR (STARP) (Long et al. 2017) and Amplifluor-like (Jatayev et al. 2017) which can be used with any commercial mastermix, significantly reducing the per-data-point cost. More recently, other commercial alternatives of KASP assays were introduced, including PACE® mastermix from 3CR Biosciences (www.3crbio.com) and rhAmp from Integrated DNA Technologies® (https://www.idtdna.com/pages/products/qpcr-and-pcr/genotyping/rhamp-snp-genotyping). However, their acceptance in wheat breeding programs is yet to be seen.

4 QTL and GWAS for End-Use Quality in Wheat Using SNP Arrays

QTL mapping and GWAS have been exponentially increased in all major crops including wheat due to the introduction of high-throughput and cost-effective genotyping platforms (Rasheed et al. 2017). Due to the co-dominant nature and high abundance, SNPs are the ideal markers for QTL and GWAS studies. SNP arrays have become a cost-effective and high-throughput means for genotyping and currently several SNP arrays are available for wheat. A 90 K SNP array was developed and almost 3380 wheat accessions were characterized (Wang et al. 2014) and have been extensively used in QTL and GWAS experiments (Table 2). To overcome the several limitations in 90 K, Winfield et al. (2016) developed an 820 K Affymetrix Axiom SNP array from resequencing exomes of 43 bread wheat and wild species accessions representing the primary, secondary and tertiary gene pools. The 820 K SNP array was used to characterize 475 bread wheat and wheat relative accessions. A subset of SNPs from the 820 K array were then used to design a breeder-oriented Axiom 35 K SNP (Allen et al. 2017), which is effective in characterizing SNPs in wild relatives of wheat in a cost-effective manner (King et al. 2017). Recently, Rimbert et al. (2018) used whole-genome resequencing data from eight wheat accessions and discovered more than three million genome-wide SNPs from genic and intergenic regions that were mined for single-copy loci to design a 280 K SNP array. A 660 K SNP array developed at the Chinese Academy of Agricultural Sciences (CAAS) is currently in extensive use and have succeeded in identifying QTL for bread-making quality and kernel number (Jin et al. 2016) and constructing a high-density linkage map of *Agropyron cristatum* (Zhou et al. 2018). However, the features of this SNP array and criteria for selection of SNP markers were not revealed. More recently, we developed Wheat 50 K (Triticum TraitBreed array) and 15 K SNP arrays based on the most qualified SNPs selected from the Wheat 35 K, 90 K, and 660 K SNP chips. Around 135 and 150 functional markers, and 700 and 1000 SNPs tightly linked with known QTL are also included in the 50 K and 15 K SNP arrays, respectively. The new Wheat 50 K and 15 K SNP arrays are a significant step towards more uniform coverage of SNPs on all chromosomes, less frequency of redundant markers and cost-effective as compared to existing SNP arrays.

The above-mentioned arrays are useful tools for gene mapping. A brief summary of QTL and GWAS experiment for wheat end-use quality are presented in Table 2. Although genetic architecture of end-use quality in wheat has been reported using gene mapping strategies, the wide array of QTL identified have been rarely used in wheat breeding. Jin et al. (2016) identified QTL for processing quality in a recombinant inbred line (RIL) population from the Gaocheng 8901/Zhoumai 16 cross using Wheat 90 K and 660 K SNP arrays. Composite interval mapping identified 119 additive QTL on 20 chromosomes except 4D; among them, 15 accounted for more than 10% of the phenotypic variation across two or three environments. Twelve QTL for Mixograph parameters, 17 for RVA parameters and 55 for Mixolab parameters were new. Eleven QTL clusters were identified. Zhai et al. (2016) identi-

Table 2 A brief description of QTL mapping, genome-wide association studies and genomic selection in wheat for end-use quality

Study	Trait	Panel/population	Marker	QTL or selection accuracy	Reference
QTL mapping	End-use quality and mixograph	WCB414/SS RILs	DArT	19 QTL	Echeverry-Solarte et al. 2015
	Milling and baking quality	Several populations	DArT	75 QTL	Cabrera et al. 2015
	Mixograph and Mixolab	Gaocheng 8901/ Zhoumai 16 RILs	90 K and 660 K SNP array	119 QTL	Jin et al. 2016
	Arabinoxylan contents	PH82–2/Neixiang 188 RILs	SSRs/STS	15 QTL	Yang et al. 2016
	Processing quality	Ning7840/Clark RILs		41 QTL	Li et al. 2017
	Flour color	Gaocheng 8901/ Zhoumai 16 RILs	90 K SNP array	46 QTL	Zhai et al. 2016
	Processing quality	RAC875/Kukri	DArT		Maphosa et al. 2013
	Dough rheology	Drysdale/Gladius RILs		5 QTL	Maphosa et al. 2015
	Flour quality	Two RILs	DArT and SSRs	20 and 34 QTL	Deng et al. 2015
	Dough rheology	HI977/HD2329 RILs	SSRs	16 QTL	Prashant et al. 2015
GWAS	GPC and sedimentation volume	192 bread wheat lines	90 K SNP array	30 QTL	Liu et al. 2017a
	Flour related traits	469 bread wheat lines	90 K SNP array	105 QTL	Jernigan et al. 2017
	Flour color traits	166 bread wheat lines	90 K SNP array	32 QTL	Zhai et al. 2018
	Flour color traits	205 bread wheat cultivars	90 K SNP array	94 QTL	Jiang et al. 2018
	Vitamins B1 and B2	166 bread wheat lines	90 K SNP array	24 QTL	Li et al. 2018
	Strach granule size	166 bread wheat lines	90 K SNP array	48 QTL	Li et al. 2017
	Dough rheology and Alveograph	120 elite lines	DArT	20 QTL	Tadesse et al. 2015
Genomic selection	Bread-making quality	5520 advanced lines	GBS	0.32 to 0.62	Battenfield et al. 2016
	Bread-making quality	6095 advanced lines	GBS	0.52–0.93	Lado et al. 2018

(continued)

Table 2 (continued)

Study	Trait	Panel/population	Marker	QTL or selection accuracy	Reference
	Bread-making quality	Two bi-patental soft winter wheat populations	DArT and SSRs	0.42–0.66	Heffner et al. 2011
	Bread-making quality	840 winter wheats	DArTseq	0.38–0.63	Michel et al. 2018
	End-use quality	398 wheat lines	90 K SNP array	0 to 0.69	Hayes et al. 2017
	Grain yield and quality	170 cultivars and mapping population	90 K SNP array	0.-0.8	Haile et al. 2018
	End-use quality	635 winter wheat lines	15 K illumina array	0.50–0.79	Kristensen et al. 2018

fied 56 QTL for flour color-related traits and PPO activity from the same population. A GWAS experiment in 469 soft winter wheat cultivars identified 105 significant marker-trait associations for flour yield, lactic acid solvent retention capacity, flour SDS sedimentation and flour swelling volume using 90 K SNP array (Jernigan et al. 2017). QTL clusters were detected for grain quality on chromosomes 1B, 6B and 7B in a doubled haploid population CO940610/'Platte' (Dao et al. 2016). Maphosa et al. (2014) identified QTL for several bread-making quality traits including flour water absorption, protein content and dough rheology in a cross between Drysdale and Gladius. Genomic regions containing photoperiod sensitivity loci affected grain protein content while the *Ha* (puroindoline) locus on chromosome 5D was associated with loaf quality traits. Other QTL (on chromosomes 2B, 3B and 5A) were novel and not associated with any known quality or phenology genes. The new loci identified using GWAS approach need to be further validated in bi-parental populations before using in marker-assisted selection.

Arabinoxylans (AX) are major polymers of wheat grain cell walls and affect the end-use properties and nutritional quality. Yang et al.(2016) identified two pairs of epistatic QTL for AX in the RILs derived from the cross PH 82–2/Neixiang 188. Additionally, a QTL on chromosome 1B likely to be the 1B.1R translocation showed stable effects on AX contents across seasons. In addition, several GWAS studies have been conducted in Chinese wheats to underpinning the genetic basis of end-use quality (Marcotuli et al. 2015). More than 50 SNP markers were associated with grain protein contents and SDS sedimentation volume in 192 bread wheat lines from China genotyped with 90 K SNP array (Liu et al. 2017a). Similarly, 205 elite wheat cultivars genotyped with 90 K SNP array were used to identify 28, 30, 24 and 12 marker-traits associations for L∗, a∗, b∗ traits and PPO activity. They found that a SNP within the *Pina-D1* was associated with all the color-related traits in wheat. Two GWAS experiments in 166 wheat cultivars and elite lines from China identified the marker-trait associations for starch granule size distribution (Li et al. 2017) and vitamins B1 and B2 using 90 K SNP array (Li et al. 2018).

Another significant quality limiting factor is the black point reaction in wheat. Black point is characterized by discoloration at the embryo end of kernels and downgrade end-use quality of the grain by discoloration. It is a serious problem in China, USA, Australia, Canada and Siberia. There is a huge knowledge gap on the actual cause of black point in wheat and the genetic basis of tolerance to black point reaction. Liu et al. (2016) identified 9 QTL for black point resistance in wheat using a RIL population derived from Linmai 2/Zhong 892 cross. Similarly, a GWAS for black point resistance was conducted in 166 diverse wheat cultivars mainly from China and 25 loci associated with black point resistance were identified (Liu et al. 2017b). These two studies provide novel insight into genetic architecture of black point resistance in wheat and the tightly linked SNP markers can be used in QTL and GWAS for black point resistance could be used for marker-assisted selection.

Although there are increasing numbers of reports to unravel the genetic architecture of wheat end-use quality using gene mapping approaches, the downstream translation of marker development to be used in wheat breeding is extremely slow.

5 Development of Wheat Cultivars With Desirable End-Use Quality

Development of the high-yield cultivars has always been the top priority worldwide, however quality has become an increasingly important objective over the years even in China and India. Gene-specific markers for quality traits as listed in Table 1, have been extensively used globally, including CIMMYT and CAAS, particularly for characterizing crossing parents and confirming the presence of targeted genes for advanced lines. Actually, improvement of quality and disease resistance such as for rusts and powdery mildew are the best examples of application of molecular markers in developing cultivars.

Improvement of dough strength and color related traits are major breeding objectives in China. Gene-specific markers and quality testing have been fully integrated into our breeding programs. Our strategies include (a) selection of crossing parents based on quality testing as well as genotype data from molecular markers, (b) limited backcross or single cross approach depending on the agronomic performance of both parents, (c) large population size such as 500–600 plants in BC_1(backcross 1), (d) selection of plants based on agronomic performance and molecular marker testing as well as quality testing including SDS sedimentation value or Mixograph data in segregating populations, and (e) yield and adaptation testing as well as quality evaluation and confirmation of presence of targeted genes by molecular markers. Seven quality cultivars such as Zhongmai 996, Zhongmai 998, Zhongmai 1062, Jimai 23, Zhongmai 29, and Zhongmai 578, have been released in various provinces of China by this approach. The molecular markers used included these for *PPO*, *PSY1*, *Glu-1* and *Glu-3* genes (He et al. 2014). Our experiences indicate that molecular markers can significantly improve breeding efficiency since only 5% of our crosses is targeted by molecular marker-assisted selection (MAS). Improvement of dough strength and

color is relatively easy to achieve, however improvement of dough extensibility is more challenging, thus at least one crossing parent must confer outstanding extensibility. The other 95% of crosses are still managed by conventional breeding since no gene-specific marker is available for targeted traits such as yield and adaptation.

6 Role of Gene Editing for Quality Improvement

The recent advances in molecular genetics have made it possible to edit specific genes based on site-specific nucleases. This offers exciting potential to precisely edit targeted important genes with greater speed and accuracy. The extensive studies involving clustered regulatory interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) have been tested in several commercial crops (Lozano-Juste and Cutler 2014). The CRISPR/Cas9 is robust, affordable and easy to use and shown to be useful across a range of plant species (Jiang et al. 2013). Although, reports on the use of CRISPR/Cas9 are limited, it is expected that large-scale germplasm-characterization efforts in conjunction with CRISPR-based genome-editing technologies will herald a new era whereby crop plants can be precisely modified without necessarily use of physical seed samples that contain important traits. Towards wheat quality improvement, Sánchez-León et al. (2018) reported a successful modification of a gene related to end-use quality. They demonstrated that CRISPR/Cas9 modified the coeliac disease causing α-gliadin gene array and obtained non-transgenic, low–gluten wheat lines. Previously, traditional mutagenesis was failed to achieve low–gluten wheat due to the complexity of the *Gli*–2 locus and the high copy number of the α–gliadin genes. However, CRISPR/Cas9 efficiently and precisely targeted the conserved regions of the α–gliadin genes in bread and durum wheat, leading to high–frequency mutagenesis in most gene copies. Although, both CRISPR/Cas9 and RNAi are highly effective for obtaining wheat lines lacking coeliac disease epitopes, the CRISPR/Cas9 has the advantages of inducing stable and heritable mutations that do not involve the expression of a transgene, and also provide a phenotype that is independent of environmental conditions. It is expected the CRISPR/Cas9 and its various new variants would be an exceptional robust tool to precisely manipulate the functional genes underpinning wheat bread-making quality.

7 Genomic Selection for Quality Improvement

As mentioned above, past selection processes in wheat breeding relied on phenotypic traits that historically led to a non-steady rate of genetic gain in breeding. Genomic selection (also referred to genomic prediction) or genome-wide selection (GS) has emerged as a strategy extensively used in animal breeding to steadily achieve genetic gain. It has also shown significant outcomes in crop breeding (Bernardo 2016) in both pure line breeding and hybrid breeding (Crossa et al. 2017).

In GS, a test population representing the genetic diversity of a large breeding population is thoroughly genotyped and phenotyped to predict phenotypic performance based on genomically estimated breeding values (GEBVs). The large breeding population is then genotyped and the GEBVs are used to predict the phenotypes of lines in the population. According to Hickey et al. (2017), GS directly addresses four factors that affect the rate of genetic gain: (i) the speed of GS should be faster than phenotypic selection and breeders can recycle genotypes more quickly, (ii) selection intensity is greater than phenotypic selection and more individuals can be selected based on GEBVs, (iii) GEBVs are more accurate than estimated breeding values based on phenotype and pedigree alone, and (iv) GS can more efficiently integrate wide crossing and pre-breeding.

GS has emerged as a valuable tool for improving complex traits controlled by QTL with small effects. Various simulation models for predicting selection accuracy depend largely on marker density, marker type, size of training populations, and trait heritability. Due to its promise, GS has been practiced extensively in wheat breeding. GS has not only been applied to bread wheat cultivars to predict grain yield (Belamkar et al. 2018), disease resistance (Juliana et al. 2017), and end-use quality (Hayes et al. 2017), but also in wheat genetic resources to predict breeding value. GS has great potential for improving bread-making and end-use quality because most of the quality testing is laborious, time-consuming, costly, need large amount of seed and destructive in nature. Michel et al. (2018) tested more than 400 wheat accessions for protein content, dough viscoelastic and mixing properties related to baking quality, and predicted genomic selection accuracy between $r = 0.39–0.47$ for these traits. They postulated that GS can be applied 2–3 years earlier than direct phenotypic selection, and the estimated selection response was nearly twice as high in comparison with indirect selection by protein content for baking quality related traits. This considerable advantage of genomic selection could accordingly support breeders in their selection decisions and aid in efficiently combining superior baking quality with grain yield in newly developed wheat cultivars.

Previously, Heffner et al. (2011) conducted first genomic selection experiment in two soft winter wheat bi-parental populations. The prediction accuracy was greater than MAS for all the traits and the average ratio of GS accuracy to phenotypic selection accuracy was 0.66, 0.54, and 0.42 for training population sizes of 96, 48, and 24, respectively. These results provide further empirical evidence that GS could produce greater genetic gain per unit time and cost than both phenotypic selection and conventional MAS in plant breeding with use of year-round nurseries and inexpensive, high-throughput genotyping technology. Hayes et al. (2017) derived NIR and NMR predictions for 19 end-use quality traits in 398 wheat accessions and predicted selection accuracy in 2420 wheat accessions. The accuracy ranged from 0 to 0.47 before the addition of the NIR/NMR data, while after these data were added, it ranged from 0 to 0.69. Genomic predictions were reasonably robust across locations and years for most traits. Using NIR and NMR predictions of quality traits overcomes a major barrier for the application of genomic selection for grain end-use quality traits in wheat breeding.

The genomic selection prediction models were tested in CIMMYT bread wheat breeding program for end-use quality phenotypes. Battenfield et al. (2016) characterized 5520 breeding lines for basic quality parameters including flour yield, protein content, SDS-sedimentation and Mixograph and Alveograph performance. The prediction accuracy ranged from 0.32 (grain hardness) to 0.62 (mixing time). Similarly, two bread wheat populations, 1465 spring wheat lines from Uruguay and 6095 lines from CIMMYT, were used to predict the quality performance of progenies from single crosses. Overall, GS appeared to be a promising tool to facilitate the early generation selection for end-use quality in wheat and higher rates of genetic gain could be possible in bread wheat. Compared with QTL mapping and GWAS, GS has more promise in harnessing genetic gains from genetic resources for quantitative traits and is seen as a more reliable and useful approach (Bernardo 2016). However, the key challenges in successful practice of GS depend on cost-effectiveness and less biased approaches for genotyping, software for handling, quality control and joint analysis of genotypic, phenotypic and environment data, and a streamlined work flow for using GS within the overall breeding pipeline.

8 Conclusion and Future Prospects

Wheat end-use quality traits are difficult to breed because their phenotypic evaluation is costly, time-consuming and labor intensive. Furthermore, phenotyping for quality traits is only possible in the late breeding cycles due to the large amount of sample requirement and destructive nature of phenotyping assays. Therefore, these traits are ideal targets for marker-assisted selection or genome-wide selection. A major fraction of genes responsible for bread-making quality is known and their functional markers are available. Such genes could be easily deployed in breeding programs through MAS. Recently, a major barrier was overcome in practicing MAS by developing high-throughput KASP markers for several important wheat end-use quality traits and it is now possible to screen thousands of wheat accessions for major genes in a day. The successful use of CRISPR/Cas9 to desirably edit the functional genes indicated that future strategies can be designed in using molecular marker in the context of gene-editing to fine tune allelic effects of genes on major quality traits.

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