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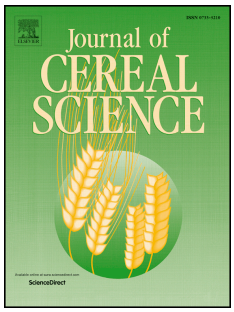

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Allelic effects and variations for key bread-making quality genes in bread wheat using high-throughput molecular markers

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Abstract

We developed and validated high-throughput Kompetitive Allele-Specific PCR (KASP) assays for key genes underpinning bread-making quality, including the *wbm* gene on chromosome 7AL and over-expressed glutenin $Bx7^{OE}$ (*Glu-B1al*) genes. Additionally, we used pre-existing KASP assay for *Sec1* (1B.1R) translocation on chromosome 1B. The newly developed KASP assays were compared with gel-based markers for reliability and phenotypically validated in a diversity panel for Mixograph, Rapid Visco Analyzer (RVA) and Mixolab traits. Genotypes carrying the 1B.1R translocation had significantly lower Mixolab parameters than those without the translocation. Similarly, superior allelic effects of the *wbm+* and $Bx7^{OE}$ alleles on Mixograph and RVA properties and their extremely low frequencies in global wheat collections supported the idea of using these genes for bread-making quality improvement. The allelic frequencies of *wbm+* and $Bx7^{OE}$ were extremely low in historical Chinese and CIMMYT wheat germplasm, but were relatively higher in synthetic hexaploid wheats and their breeding derivatives. In both the Vavilov and Watkins global landrace collections, the frequency of *wbm+* was 6.4 and 3.5%, and frequency of $Bx7^{OE}$ was 3.2% and 7.0%, respectively. The high-throughput marker resources and large-scale global germplasm screening provided further opportunities to exploit these genes in wheat breeding to enhance bread-making quality.

Keywords: $Bx7^{OE}$, 1B.1R translocation, KASP markers; Mixolab, Mixograph; Rapid visco analyzer

Introduction

Bread-making quality is an important breeding objective due to the reliance of one-third of the global population on wheat and wheat products for food and calories. Genetic variation in bread-making quality of wheat depends on the viscoelastic properties of dough, largely underpinned by high-molecular weight (HMW-GS) and low-molecular weight (LMW-GS) glutenin subunits (Rasheed et al., 2014b). The HWW-GS genes are *Glu-A1*, *Glu-B1* and *Glu-D1* loci located on the long arms of group 1 chromosomes. Each gene encodes an x-type and y-type subunit, giving rise to an extensive allelic series in wheat and its close relatives. Several alleles at these loci confer superior bread-making quality attributes that are important breeding targets in producing new cultivars. For example, the *Glu-D1d* allele (subunits Dx5+Dx10) is associated with superior mixing strength and bread-making quality compared to *Glu-D1a* (subunits Dx2+Dy12) (Rasheed et al., 2016; Zheng et al., 2009). Similarly, the *Glu-B1al* allele (subunits Bx7^{OE}+By8) at *Glu-B1* is associated with enhanced dough strength over the more common *Glu-B1b* allele (subunits Bx7+By8) (Butow et al., 2003; Cooper et al., 2016). This superiority of the *Glu-B1al* allele (hereafter *Bx7^{OE}*) is a consequence of over-expression of the x-type subunit due to the segmental duplication of a 10.3 kb region that includes the *Bx7* allele (Ragupathy et al., 2008). Screening of tetraploid and hexaploid wheat cultivars revealed very low frequencies of *Bx7^{OE}* indicating opportunities to exploit this allele in breeding (Ragupathy et al., 2008).

The most significant measurable output from tertiary germplasm sources was the fortuitous spontaneous wheat-alien chromosome translocation, 1BL.1RS, that occurred in the 1930s, but greatly contributed to world wheat production in various countries, but especially after its release in CIMMYT varieties from the mid-1970's,

initially with VEERY “S”. Varieties carrying the 1BL.1RS translocation occupied more than 50% of the wheat area in China in the 1990s and are still cultivated on about 25% of the current production area. The 1BL.1RS translocation was initially used for winter wheat in Europe and the former USSR. China then used it in winter wheats, and CIMMYT developed spring growth habit derivatives. However, the 1BL.1RS translocation encodes the *Sec-1* gene, in addition to several other biotic stress resistance genes, that causes undesirable dough stickiness under some circumstances. This gene is gradually being deselected in breeding programs. Therefore, marker-assisted screening for the 1BL.1RS translocation in breeding programs remains a routine exercise.

Knowledge on genetic control of gluten strength and elasticity beyond the prolamins (glutenins and gliadins) is largely unknown and restricted to a few major QTLs. Recent advances in molecular genetics provide new opportunities to identify the genetic basis of bread-making quality in wheat beyond the prolamins. 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 varieties with good bread-making quality. Guzman et al. (2016) later identified 8 of 56 CIMMYT varieties 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.

Kompetitive allele-specific PCR (KASP) markers offer high-throughput screening with scalable flexibility, and are the preferred markers for large-scale screening of germplasm. Recently, we developed and validated more than 70 KASP marker for

functional genes in wheat (Rasheed et al., 2016). This study is a continuation of a previous effort to develop KASP markers for very important wheat quality related genes. The objectives of the current study were to i) develop high-throughput KASP markers for wbm, *Glu-B1al* and 1B.1R in wheat, ii) assess the allelic effects of these genes in a wheat diversity panel, and iii) screen large collections of wheat landraces, cultivars and advanced breeding lines to identify superior allelic sources of these genes.

Experimental

Germplasm

A wheat diversity panel comprising 305 varieties was used to assess the allelic effects of the *wbm*, *Bx7*^{OE} and *Sec-1* genes in wheat using newly developed KASP markers. This panel included 223 historical and current wheat varieties widely grown in the Yellow and Huai River valleys and Yangtze region in China, and 77 cultivars from 13 countries including the USA, Australia, Japan, and Europe. This panel was extensively phenotyped for quality traits (Rasheed et al., 2016; Jin et al., 2017). The allelic frequencies of these genes were also determined in more than 1,200 landraces and 2,000 modern varieties, including 439 accessions from the Watkins global landrace collection (Wingen et al., 2014), 292 accessions of the Vavilov diversity panel (Riaz et al., 2017), 94 accessions of the global tetraploid wheat collection (Ren et al., 2013), 182 accessions from *T. turgidum* landraces belonging to ssp. turanicum (n=2), cathlicum (n=43), dicoccon (n=4), durum (n=136), polonicum (n=3), turgidum (n=4), 2,000 cultivars and advanced lines from different wheat breeding programs in China, 284 historical CIMMYT accessions (Lopes et al., 2015), 340 European winter wheat cultivars (Kollers et al., 2013), a 182 accessions from USA, 52 Australian

cultivars, 209 lines in a synthetic derived diversity panel (Afzal et al., 2017), and 127 lines in a primary synthetics diversity panel (Rasheed et al., 2014a).

Phenotyping

Field trials of the diversity panel were conducted at Anyang (Henan province) and Beijing during the 2012-2013 and 2013-2014 cropping seasons. All field trials were planted in randomized complete blocks with three replications. Plots consisted of four 1.5 m rows with 20 cm between rows. Approximately 50 seeds were sown in each row.

Grain of the diversity panel from each site and year were used for quality analysis. Kernel hardness and moisture were determined using a Single Kernel Characterization System (SKCS 4100, Perten, Sweden). Samples were tempered overnight to 15.5 and 16.5% moisture contents for medium (SKCS hardness index, 40-59) and hard (SKCS hardness index, > 60) textured types, respectively. All samples were milled on a Buhler MLU 202 laboratory mill (Buhler Bros, Ltd, Uzwil, Switzerland) based on AACC (2000) method 26-21A to produce flour with straight-run extraction rates. A 10 g mixograph (National Mfg. Co., Lincoln, NE) was used to evaluate the mixing properties of the flour samples. Midline peak time (MPT), midline peak height (MPH); midline peak width (MPW), and midline time x = 8 width (MTxW) were recorded. A RVA (Newport Scientific, Australia) was employed to evaluate the starch pasting properties of flour samples and PV, trough viscosity (TV), BD, final viscosity (FV), setback (SB), and peak time (PTI) were scored following AACC (2000) method 76-21 with minor modifications, viz., the reaction solution of water was replaced by 170 mg/L $AgNO_3$ to eliminate the effect of α-amylase activity in flour on starch pasting properties.

A Mixolab (Chopin Technologies, France) was used to determine dough mixing and pasting properties of wheat flour simultaneously during dough mixing. About 50-g of flour was put into the Mixolab bowl and an appropriate amount of water was added to ensure that the torque of the dough was in the 1.1 ± 0.07 Nm range. Processing was divided into five stages based on the “Chopin 12heat” protocol as follows:

establishing equilibrium at 30°C for 8 min, then heating to 90°C at a rate of 4°C/min for 15 min, holding at 90°C for 12 min, cooling to 50°C at a rate of 4°C/min for 10 min, and finally holding at 50°C for 5 min. The mixing speed was kept constant at 80 rpm. The parameters water absorption (WA), development time (DT), stability time (ST), C1 (the torque of maximum point in the first mixing stage), C2-C5 (the torque of end points in the corresponding mixing stages) were recorded during the procedure.

Development of KASP assay for the wbm gene and genotyping

The cDNA sequence of *wbm* and genomic DNA sequence of *Bx7*^{OE} (NCBI accession number, EU157184) were retrieved and used as queries to blast in the wheat genome survey sequence database (IWGSS). The resulting contigs were aligned with query sequences to identify polymorphic sites, which were then used to develop allele-specific primers, which were designed to carry the standard FAM (5' GAAGGTGACCAAGTTCATGCT 3') and HEX (5' GAAGGTCGGAGTCAACGGATT 3') tails. The KASP marker for 1B.1R translocation (1B.1R_6110) was used from our previous report (Rasheed et al. 2016). The primer mixture comprised 46 µl ddH_2O, 30 µl common primer (100 µM) and 12 µl of each tailed primer (100 µM). Assays were tested in 384 well formats and set up as ~3 µl reactions (10-20 ng/µl dry DNA, 3 µl of 1XKASP master mixture, and 0.056 µl of primer mixture). PCR cycling was performed using the following protocol: hot start at 95°C for 15 min, followed by ten touchdown cycles (95°C for 20 s; touchdown at 65°C initially and decreasing by -1°C per cycle for 25 s), followed by 30 additional cycles of annealing (95°C for 10 s; 57°C for 60 s). An extension step was unnecessary as the amplicons are usually less than 120 bp. Genotyping was carried out in SNPline® at Huazhi Rice Research Institute, Changsha, China.

Statistical analysis

Allelic effects were estimated using PROC MIXED in the Statistical Analysis System (SAS Institute, 2000) for all quality-related traits, with genotypes and alleles considered as random effect. Significant differences between allelic groups were estimated using adjusted least square mean (LSM) analyses with a threshold probability of $P < 0.05$ in Student's t-tests.

Results

Development of KASP assays for genes

Blast analysis identified polymorphic sites between alleles, which were then used to design primers (Figure 1a, b, c). For the *wbm* gene, there were five SNPs between *wbm+* and *wbm-* alleles, allowing design of two allele-specific primers (Figure 1a) and one common primer covering a total 44 bp segment. For $Bx7^{OE}$, we first tried the primer sites of two STS markers from Ragupathy et al. (2008). But these KASP assays were not successful and did not gave consistent results. Therefore, an allele-specific and common primers were designed from polymorphic sites between $Bx7^{OE}$ BAC library (EU157184) and Bx7 alleles. This allowed amplification of the $Bx7^{OE}$ allele (Figure 1b) on the FAM axis. The second allele-specific primer from the same site failed to amplify the contrasting allele (non-$Bx7^{OE}$) possibly due to common polymorphisms in varieties with contrasting alleles. Therefore, a more conserved region was selected to design another pair of primers (allele-specific and reverse) amplified as a housekeeping control on the HEX axis; there was amplification by all varieties (Table 1). The results from KASP markers were compared to contrasting gel-based markers for all three genes, and there was 100% consistency for the *wbm*

gene. However, consistency of the *Bx7*^{OE} marker was about 95%, with two false positive among 48 accessions.

Allelic effects of genes and variation in the diversity panel

Only six varieties in the diversity panel had the *Bx7*^{OE} allele (namely, Dorico from Italy, ProINTA Colibr 1, Klein Jabal 1 from Argentina, Insignia from France, Jingdong 17 and Qinnong 151 from China) and six (Mantol from Italy, Aca 601 from Argentina, Insignia from France, Manital from France, Kiniish 46 from Russia, and Jinmai 67 from China) had the *wbm+* allele. Only Insignia had both the *wbm+* and *Bx7*^{OE} alleles. Since the frequencies of the two genes were very low their allelic effects were compared to the varieties in diversity panel having Ax1 or Ax2* at *Glu-A1* and 5+10 at *Glu-D1*. All starch pasting properties measured by rapid visco analyzer (RVA) were significantly lower in *wbm+* genotypes compared to *wbm-* genotypes, while Mixograph properties were significantly higher in *wbm+* genotypes (Table 2). The bread-making profile of two closely related Chinese wheat cultivars Mianmai 37 (*wbm-*) and Mianmai 1419 (*wbm+*) significantly differed for dough extensibility and loaf volume (Figure S1). The alleged donor for *wbm+* in Mianmai 1419 is likely a CIMMYT advanced line CIMY09A455 (KIRITATI//2*PBW65/2*SERL1B). All *Bx7*^{OE} genotypes had significantly lower RVA parameters except FV, while all Mixograph properties were significantly higher compared to *non-Bx7*^{OE} genotypes. In diversity panel, 166 accessions were phenotyped for Mixolab parameters, of which 64 carried the 1BL.1RS translocation. All Mixolab parameters were significantly lower in 1BL.1RS genotypes except for water absorption (WA), which was non-significant (Table 3).

Frequencies of genes in global wheat germplasm

The frequencies of *wbm+*, $Bx7^{OE}$ and 1B.1R in the global wheat collections are provided in Table 4 and a detailed list is provided in Table S1. Only four of the 2,000 Chinese accessions carried the *wbm+* gene; three (Chuanmai-38, Chuanmai-82, 14046) were from Sichuan and one (Winter No.21) was from Xinjiang province. Similarly, screening of the CIMMYT historical wheat variety set referred to as 'WAMI' (Wheat Association Mapping Initiative) detected only 10 accessions carrying the *wbm+* gene (Table S1). No tetraploid wheat accession carried *wbm+*, and only three (PI 520415 from Syria, PI 546060 from Canada, PI 352389 from Greece and 127109 from Russia) carried $Bx7^{OE}$ allele. Eighteen accessions in Vavilov diversity panel carried *wbm+* gene, however the geographic information of 11 accessions are unknown. 16 out of 18 *wbm+* accessions from Vavilov's panel had spring growth habit and only two had winter growth habit (Table S1).

Discussion

Discovery of new genes associated with bread-making quality and MAS of favorable alleles in breeding programs is critical for ongoing improvement of end-use quality of wheat. The deployment of favorable alleles in improved cultivars could be accelerated if sources of the alleles are known in various genetic backgrounds and efficient molecular diagnostics are available for gene introgression (Rasheed et al., 2017). We used KASP markers for three important genes related to bread-making quality and their allelic effects on Mixograph and RVA properties were validated. For the first time the effect of the 1BL.1RS translocation was also validated based on Mixolab properties. Mixolab is a relatively new tool for quality analysis and can be used to predict bread wheat quality and to differentiate wheat genotypes in terms of different quality characteristics (Koksel et al., 2009). Jin et al. (2016) found positive

correlations between all Mixolab parameters and Mixograph parameters and negative correlation between WA and other Mixolab parameters in a Gaocheng 8901/Zhoumai 16 RIL population. Our results confirmed that the presence of 1B.1R leads to significantly lower protein quality related (C2) and starch gelatinization related (C3, C4 and C5) parameters with no effect on water absorption. Previously, Chen et al. (2013) assessed allelic effects of the *Pina-D1* and *Pinb-D1* alleles on Mixolab parameters and found that *Pina-D1a/Pinb-D1b* genotypes had lower water absorption and C2 values but higher C3, C4 and C5. There is limited literature on the allelic effects of other quality related genes due to the recent development and adoption of this equipment, and our results provide a strong basis for comparison in future studies because we used a large diversity panel for comparison.

The extremely low frequencies of the *wbm+* and $Bx7^{OE}$ alleles in improved germplasm highlights the opportunity to manipulate and deploy these alleles to improve bread-making quality attributes of wheat varieties grown worldwide. The predicted wbm protein, which is sulphur rich, suggests the possibility of a contribution to bread loaf volume by supporting the crossing linking of proteins in gluten. The allelic effect of *wbm+* was in agreement with Guzman et al. (2016) and Furtado et al. (2015) in showing that despite non-significant changes in protein content, all other Mixograph parameters were significantly higher. The gene was identified recently and its frequency was determined in only a small set of CIMMYT germplasm and in Australian wheat cultivars (Furtado et al., 2015; Guzman et al., 2016). A slightly higher frequency of *wbm+* in synthetic hexaploid wheat lines and their advanced derivatives indicated the gene can be readily introduced using various synthetic wheat sources. As this gene is located on chromosome 7A, its source in synthetic wheat accessions was the durum parents. However, none of the global

tetraploid accessions screened in our study carried the gene, suggesting it may be common only in improved durum lines. Despite the low frequency of *wbm+* in our global durum panel it was present in almost all genetic backgrounds. Allele *wbm+* was identified in only Chinese accessions and these came from from Xinjiang and Sichuan provinces, which cultivate spring wheats. The likely source was CIMMYT germplasm.

The allelic effect of $Bx7^{OE}$ was similar to *wbm+* except RVA setback (Sb) and final viscosity (FV) were higher in accessions with $Bx7^{OE}$. The results were agreement to Cooper et al. (2016) who reported that NILs carrying $Bx7^{OE}$ consistently showed greater midline peak widths than those carrying the *Glu-B1b* allele. The frequency $Bx7^{OE}$ was also very low in the global germplasm collection; however, three tetraploid accessions carried it. These were from Switzerland (T357; PI 352377), Syria (Syrian durum 27; PI 520415) and Canada (DT367; PI 546060). We did not find this gene among the 209 synthetic-derivatives, but it was detected in 19 primary synthetic hexaploid wheat lines. Previously, none of 55 European wheat cultivars (Ragupathy et al., 2008), tetraploid wheats, and synthetic hexaploid wheat lines carried the $Bx7^{OE}$ (Li et al., 2014). However, we identified the allele in nine European winter wheat cultivars and 19 synthetic hexaploid wheats, probably because we screened large numbers. In conclusion, high-throughput KASP markers were successfully developed for three key quality-related genes, which can be used to enhance selection efficiency in wheat breeding programs.

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316

317 **Conflict of interest**

318 Authors declare no conflict of interest

319

320 Figure legends

321 Figure 1. Alignment of the DNA sequences of the *wbm* gene (a) and *Glu-B1al* (b) to

322 the IWGSC contigs for development of allele-specific and common primers.

323 Supplementary file

324 Figure S1. The bread-making profiles of two closely related Chinese wheat cultivars

325 without WBM (Mianmai 37) and with WBM (Mianmai 1419).

326 Figure S2. Scatterplots of KASP assays for Bx7OE_SNP (a) and WBM_SNP (b)

327 showing distribution of wheat genotypes in FAM and HEX clusters.

Table 1. The KASP marker sequences for *wbm*, *Bx7*^{OE} and *Sec-1* developed for high-throughput screening of these genes in wheat.

Gene	Marker	Probe	Sequence (5' - 3')	Report
wbm^{+}	WBM_SNP	FAM	GAAGGTGACCAAGTTCATGCTAATAAGGGCCGCTGCTATATGC	wbm+
		HEX	GAAGGTCGGAGTCAACGGATTAATAAGGGCTGCTACTATGTAT	wbm-
		Common	TTGCTAAAAGCAAATGGCCTGC	
Sec-1 (1B.1R)	1B1R_6110	FAM	GAAGGTGACCAAGTTCATGCTGGAGCAGGTCCAGATCGCG	1B.1R-
		HEX	GAAGGTCGGAGTCAACGGATTCGGAGCAGGTCCAGATCGCA	1B.1R+
		Common	GAAGCTCCGGTAGATGGAGGCTA	
Glu-B1al	Bx7OE_SNP[1]	FAM	GAAGGTGACCAAGTTCATGCTGCCTAGAGCCCATCTCAGATTTCAGC	Bx7^{OE}+
		HEX	GAAGGTCGGAGTCAACGGATTGGTCGATGAGATCCCGTACA	Bx7^{OE}-
		Bx7OE_R	GTTCTTCTATGATCTCCAAGTGTTTC	
		HK_R	CGAGCTAGGGTTTGTTGCGAGC	

[1]HEX and HK_R amplify a house-keeping gene in wheat, while FAM and Bx7OE_R are allele-specific for *Glu-B1al* (Bx7^{OE}) allele.

Table 2. Allelic effects of three quality related genes based on adjusted least square means of Mixograph and RVA properties in the wheat diversity panel

Gene	Allele	Frequency	GPC	FPC	HARD	PV	TV	Bd	FV	Sb	MPT	MPV	MPW	MTxW
Sec-1	1B.1R+	96	12.98	11.89	55.11	3219.73	2319.97	899.77	3906.11	1586.14	3.00	46.65	17.34	5.55
	1B.1R-	209	12.93	11.73	49.99	3282.77	2408.97	873.80	3906.40	1497.43	3.53	47.64	19.74	8.35
	t-test		ns	ns	***	***	**	***	ns	***	***	**	***	***
wbm	*wbm+*	6	14.00	12.88	73.60	3119.45	2268.37	851.08	3779.54	1511.17	4.85	49.28	22.35	13.38
	wbm-	21	13.46	12.41	61.42	3314.32	2425.49	888.83	3947.29	1521.80	4.56	47.44	19.68	10.73
	t-test		ns	ns	***	***	***	***	***	**	***	***	***	***
Glu-B1al	$Bx7^{OE}$+	6	13.67	12.59	70.86	3264.57	2397.99	866.58	3954.52	1556.53	4.85	50.99	20.66	11.84
	$Bx7^{OE}$-	20	13.51	12.44	62.07	3320.80	2433.63	887.16	3947.41	1513.78	4.65	47.43	19.74	11.11
	t-test		ns	ns	***	***	***	***	***	***	***	***	***	**

GPC: grain protein contents, **FPC**: flour protein contents, **HARD**: grain harness, **PV**: RVA peak viscosity, **TV**: RVA trough viscosity, **Bd:** RVA breakdown, **FV**: RVA final viscosity, **Sb**: RVA setback, **MPT**: Mixograph midline peak time, **MPV**: Mixograph midline peak value, **MPW**: Mixograph midline peak width, **MTxW**: Mixograph midline 8 min band width

Table 3. Allelic effects of 1B.1R translocation based on adjusted least square means of Mixolab properties in the wheat diversity panel

Gene	Allele	Frequency	WA	DT	ST	C2	C3	C4	C5
Sec-1	1B.1R+	64	61.85	3.70	5.21	0.42	1.87	1.71	3.01
	1B.1R-	102	61.46	4.44	7.06	0.46	2.04	1.85	3.20
	t-test		ns	***	***	***	***	***	***

WA: Mixolab water absorption, **DT:** Mixolab development time, **C2:** Mixolab protein weakening torque, **C3:** Mixolab starch gelatinization peak torque, **C4:** Mixolab starch gelatinization trough torque, **C5:** Mixolab starch gelatinization final torque

Table 4. Allele frequencies of the *wbm*$^{+}$, *Glu-B1al* and *Sec1* alleles in worldwide wheat collections based on newly developed KASP markers. See Table S1 for detailed list

		Frequency		
Collection	Total	*wbm+*	*Glu-B1al*	1BL.1RS
Chinese germplasm	1840	5	7	584
Chinese Mini core collection	267	Nil	1	40
CIMMYT WAMI panel	284	3	17	58
European winter wheats	273	8	Nil	94
Global tetraploid panel	94	Nil	3	Nil
Watkins global landraces	639	22	45	Nil
Vavilov wheat collection	277	18	9	Nil
Synthetic hexaploid wheats	127	24	19	Nil
Synthetic derivatives	209	36	-	58
CWANA region panel	288	9	13	49
USA	150	Nil	1	3

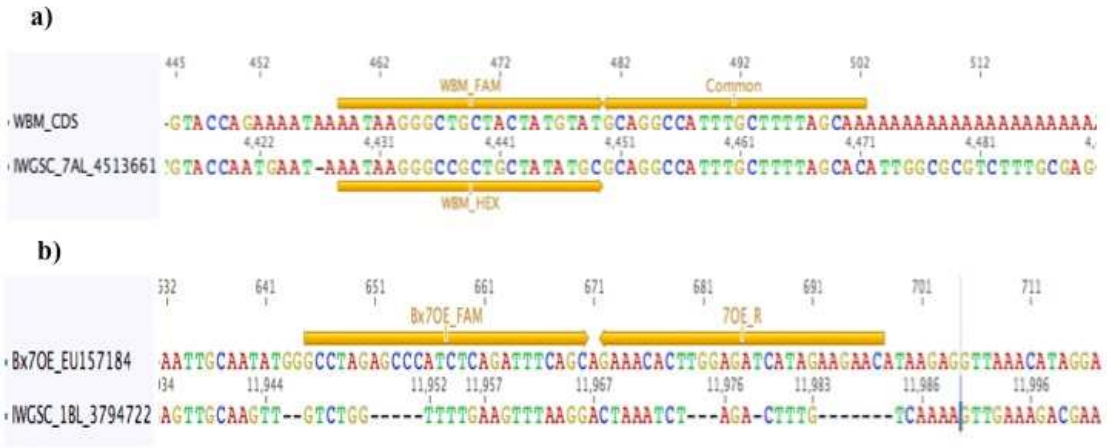

Figure 1. Alignment of the DNA sequences of the *wbm* gene (a) and *Glu-B1al* (b) to the IWGSC contigs for development of allele-specific and common primers.

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Highlights

- High-throughput KASP markers were developed for three important quality genes, *Bx7*^{OE}, *wbm* and 1BL.1RS in bread wheat.
- Significant allelic effects were identified in a diversity panel.
- Screening global landraces and cultivar collections identified candidates with superior alleles.
- The use of KASP markers could help to fine tune bread-making quality in wheat breeding.