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Identification of major loci for seed dormancy at different post-ripening stages after harvest and validation of a novel locus on chromosome 2AL in common wheat

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Abstract A long-period duration of seed dormancy helps to reduce pre-harvest sprouting (PHS) damage in common wheat when a long period of rainfall or high humidity occurs. Identification of genes or loci underlying seed dormancy duration is of high importance for investigating genetic mechanism of PHS tolerance of wheat. In the present study, the germination index (GI) of different mapping populations at 5 days (GI5), 15 days (GI15), and 30 days (GI30) after harvest (DAH) was recorded during the 2011–2012, 2012–2013, 2013–2014, and 2014–2015 cropping seasons. Field sprouting (FS) values were also determined in 2012–2013 and 2014–2015 cropping seasons. Association analysis was performed in 260 wheat varieties and advanced lines differing in seed dormancy (SD) using 557 SSR and 14 gene-specific markers for PHS/SD-related genes. The result indicated that a total of 47 loci were significantly ($P < 0.01$) associated with SD using both the general linear model (GLM) and mixed linear model (MLM). Five major loci were consistently detected at different post-ripening stages after harvest, including four for GI5 and GI15 on chromosomes 3AS, 3BL, 5AL, and 5BL responsible for middle-duration SD, and one for GI5, GI15, and GI30 on 2AL associated with longer-duration SD. The major locus on 2AL (designated *Qsd.ahau-2AL*) is likely to be a novel QTL, which was linked to *Xwmc658* by mapping analysis in 165 recombinant inbred lines (RILs) derived from the Jing 411 × Wanxianbaimaizi cross and 728 F_2 plants from Jimai 20 × Suiningtuotuomai. Furthermore, a CAPS marker *CAPS-2AL* was developed for *Qsd.ahau-2AL* locus and was validated using the 728 F_2 plants and 201 accessions of the Chinese mini-core collection. Our results also revealed that the *TaMFT-like* gene on 3AS had a more significant association with dormancy duration than the other six genes underlying

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SD/PHS resistance in Chinese wheat germplasm. This study provides useful information for marker-assisted selection in wheat breeding and enhances our understanding of molecular genetics of SD in wheat.

Keywords Association analysis · Linkage analysis · Pre-harvest sprouting · *Triticum aestivum* L

Introduction

Pre-harvest sprouting (PHS) occurs in the spikes prior to harvest and is seriously detrimental to wheat yield and quality. In China, the vast majority of wheat production areas are affected by PHS, particularly in the Middle and Lower Yangtze River Valley winter wheat region, southwestern winter wheat region, and northeastern spring wheat region (Xiao et al. 2002). This is mainly attributed to a short period of seed dormancy (SD) in wheat varieties (Chen et al. 2008; Hickey et al. 2009; Ogbonnaya et al. 2008; Fofana et al. 2009; Liu et al. 2011). PHS-resistant varieties with a high level of SD can tolerate a longer period of rainfall or high humidity, consequently reducing PHS damage. However, the genetic basis underlying SD remains elusive.

The SD trait is controlled jointly by major and minor genes. Numerous quantitative trait loci (QTLs) for SD or PHS have been identified on nearly all 21 wheat chromosomes (Gao et al. 2013). Several major QTLs have been detected on chromosomes 2BS, 3AS, 3AL, 3BL, 3DL, and 4AL in different populations (Groos et al. 2002; Mori et al. 2005; Kulwal et al. 2005; Liu et al. 2008, 2011; Chen et al. 2008; Ogbonnaya et al. 2008; Fofana et al. 2009; Munkvold et al. 2009; Jaiswal et al. 2012; Somyong et al. 2014; Lin et al. 2015; Albrecht et al. 2015; Kumar et al. 2015). Candidate genes in these regions have been identified based on comparative genomics or transcriptomic analysis, such as *TaSdr-B1* on 2BS (Zhang et al. 2014), *TaPHS1* (a *TaMFT-like* gene) on 3AS (Liu et al. 2013), *TaVp-1* and *Tamyb10* on group 3 chromosomes (Yang et al. 2007; Chang et al. 2010a, b, 2011; Himi et al. 2011), *PM19-A1* and *TaMKK3-A* on 4AL (Barrero et al. 2015; Torada et al. 2016). However, their effects on SD are not well understood because the phenotypes detected in these studies were based on GI recorded in a short-period post-maturity, rather than GI values evaluated at longer period after harvest (Groos et al. 2002; Liu et al. 2008; Singh et al. 2010; Kulwal et al. 2012). Seed germination requires a period of post-ripening to break dormancy. Thus, long-period seed dormancy is very useful for decreasing PHS damage because of long-period rainfall or high humidity during maturation of wheat. Identification of genes or loci underlying long-period seed dormancy helps to understand the genetics of PHS.

Most of loci for seed dormancy or PHS tolerance are identified by QTL mapping in previous reports. Compared with linkage mapping, in which only two alleles at each locus are evaluated, the association mapping based on linkage disequilibrium is an effective approach for identifying abundant genetic loci for complex traits (such as SD/PHS) in diverse natural populations (Rehman Arif et al. 2012; Kulwal et al. 2012; Jaiswal et al. 2012; Albrecht et al. 2015). Combination of linkage and association mapping cannot only mitigate the limitations of each but also increase the power to detect true associations and verify the results (Hendricks et al. 2009; Korir et al. 2013; Korte and Farlow. 2013). Therefore, the objectives of the present study were to (1) identify loci conferring SD at 5, 15, and 30 days after harvest (DAH) and field sprouting (FS) in a panel of Chinese wheat varieties by association analysis, (2) evaluate the effects of functional genes for seed dormancy/PHS tolerance in Chinese wheat germplasm, and (3) detect novel loci for long-period seed dormancy and validate their effects.

Materials and methods

Plant materials

Two hundred and sixty wheat varieties and advanced lines with different PHS resistance (Wang et al. 2014; Zhu et al. 2014) were used for association analysis, including 179 from Yellow and Huai Valley winter wheat region, 30 from southwestern winter wheat region, 22 from Middle and Lower Yangtze River Valley winter wheat region, 21 from northern winter wheat region, and 8 from other countries (Table S1). Two hundred and one accessions from the Chinese mini-core wheat collection were used to validate the association of a cleaved amplified polymorphic sequence (CAPS) marker (*CAPS-2AL*) for the major locus on chromosome 2AL (Table S2). One hundred and sixty-five $F_{2:8}$ RILs developed by a single-seed descent method from the Jing 411 × Wanxianbaimaizi cross, and 728 F_2 plants from the Jimai 20 × Suiningtuotuomai cross

were also used to validate the novel locus for SD on 2AL. Wanxianbaimaizi and Suiningtuotuomai are white-grained landraces with relatively high levels of SD, whereas Jing 411 and Jimai 20 are white-grained varieties with low and moderate levels of SD, respectively (Chang et al. 2010a, b, 2011, Table S3).

Field trials

The 260 wheat varieties/lines were planted at the experimental station of Anhui Agricultural University in Hefei (31°58′N, 117°240′E) during the 2011–2012, 2012–2013, 2013–2014, and 2014–2015 cropping seasons; 201 accessions of the Chinese mini-core wheat collection were grown at the experimental station during the 2013–2014 and 2014–2015 cropping seasons, and the RILs derived from the Jing 411 × Wanxianbaimaizi cross and F_2 population from Jimai 20 × Suiningtuotuomai were planted at the experimental station during the 2014–2015 cropping season. Field trials were conducted in plots with double 4-m rows and 25 cm between rows in randomized complete blocks with two replications. Field management followed local agricultural practice.

Heading date was scored when 50% of spikes in a plot were emerged. Sixty spikes of each plot were collected at physiological maturity, naturally air dried for 4 days, hand threshed to avoid damage to the embryos and seed coat, and then stored at −20 °C until all were harvested. After all lines were threshed, they were stored at room temperature (25 °C) for subsequent germination assay.

Germination index assay

Seed dormancy duration was revealed by germination index (GI) at 5, 15, and 30 DAH. The GI was assayed following Chang et al. (2010a, b). For 260 wheat varieties, GI was evaluated at 5 DAH (designated 12GI5, 13GI5, 14GI5, and 15GI5), 15 DAH (designated 12GI15, 13GI15, 14GI15, and 15GI15), and 30 DAH (designated 12GI30, 13GI30, 14GI30, and 15GI30) in 2012, 2013, 2014, and 2015, respectively. The GI of 201 accessions of the Chinese mini-core collection (CMCC) was tested at 5 and 15 DAH in 2014 and 2015 (designated 14GI5-CMCC, 14GI15-CMCC, 15GI5-CMCC, and 15GI15-CMCC, respectively). The Jing 411 × Wanxianbaimaizi and Jimai 20 × Suiningtuotuomai populations were assayed for GI at 5 and 15 DAH in 2015 (designated 15GI5-JW, 15GI15-JW, 15GI5-JS, and 15GI15-JS, respectively).

Field sprouting test

In 2013 and 2015, the rainfall occurred for over a week during the harvest season (Tables S4 and S5), resulting in severe PHS in the field. For the 260 wheat varieties, 165 RILs from Jing 411 × Wanxianbaimaizi and two parents, 10 spikes of each plot were left in the field for 7 and 8 days in 2013 and 2015, respectively, and then collected and dried immediately in an oven (105 °C for 2 h) for FS test. Sprouted grains were scored, in which the pericarp over the embryo was ruptured. The FS value was determined based on the averaged sprouted grains of 20 spikes collected from two plots of each line. The FS values of 260 wheat varieties tested in 2013 and 2015 were designated 13FS and 15FS, respectively; those of Jing 411 × Wanxianbaimaizi population were designated 15FS-JW in 2015.

Genotyping

Genomic DNA was isolated from undamaged dry kernels following Zhu et al. (2014). A total of 557 highly polymorphic SSR markers, selected from 1723 SSRs including *Xbarc*, *Xwmc*, *Xgwm*, *Xcfa*, *Xcfd*, and *Xgdm* (GrainGenes, http://wheat.pw.usda.gov/GG2/index.shtm/), were used to genotype the 260 wheat varieties. PCR was performed in a 10-μL volume following Zhu et al. (2014). To validate the genotyping results, SSR markers with abundant polymorphisms at 6% denaturing polyacrylamide gel electrophoresis (PAGE) were conducted again using Fragment Analyzer™ Automated CE System (including mixture of dsDNA 800 Separation Gel and intercalating Dye (20:1), fivefold diluted capillary conditioning solution, a deep 96-well plate of fivefold diluted 930 dsDNA inlet buffer, a 96-well plate reusable oil-sealed markers (35 and 500 bp), and a 96-well sample plate). Following the user's instructions, 3 μL of PCR products mentioned above was diluted using 20 μL of 1 × TE buffer and transferred to another 96-well sample plate (1–95th wells). The 96th well was added 75–400 bp DNA ladder as marker. After filling fresh gel in 96 capillaries and voltage separation pre-run (6.0 kV, 30s), markers and samples were voltage injected in 5.0 kV for 15 s and 9.0 kV for 15 s, respectively, and then separated in 9.0 kV for 1 h. Genotyping

was performed using ProSize 2.0 software following the manufacturer's directions (www.aati-us.com).

Fourteen gene-specific markers from seven candidate genes related to SD or PHS resistance, including *Sdr2B* for *TaSdr-B1* (Zhang et al. 2014), *TaMFT-3A*, *TaMFT-A1*, *SNP_646*, and *SNP_666* for a *TaMFT-like* gene (Nakamura et al. 2011; Lei et al. 2013; Liu et al. 2013, 2015), *Vp1B3*, *Vp1-b2*, and *A17–19* for *TaVp-1* (Yang et al. 2007; Chang et al. 2010a, 2011), *Tamyb10-A1*, *Tamyb10-B1* and *Tamyb10-D1* for *Tamyb10* (Himi et al. 2011), *TaDFR-B* for *TaDFR* (Bi et al. 2014), *PM19-A1* for *TaPM19-A1* (Barrero et al. 2015), and *MKKAC* for *TaMKK3-A* (Torada et al. 2016), were used for association analysis together with SSR markers. PCR and gel electrophoresis were performed following previous studies mentioned above except for *MKKAC*.

A dCAPS marker (designated *MKKAC*) was developed for *TaMKK3-A* using the software Primer premier 5.0 (www.PremierBiosoft.com) to genotype the 260 wheat lines based on a SNP (A/C) located at 660 bp downstream of the initiation codon (Fig. S1). The first PCR was conducted using the *TaMKK3-A* genome-specific primer *A3* (Torada et al. 2016), the PCR amplification consisted of an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 40 s at 94 °C, 40 s at 61 °C, 1 min at 72 °C, and a final step of 8 min at 72 °C. The second PCR with dCAPS primers (F: CACATCCT CTTCCTTTCA, R: TTTGCTTCGCCCTTAAC) was performed using the first PCR product as template DNA in a total volume of 10 μL, including 1.0 μL of 10 × PCR buffer, 200 μM of dNTPs, 4 pmol of each primer, 0.5 U *Taq* DNA polymerase, and 100 ng of template DNA. The PCR included an initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, 58 °C for 30 s with a decrease of 0.2 °C per cycle, and 72 °C for 30 s and a final extension at 72 °C for 10 min. Then, the second PCR product was digested with *HpyCH4*IV for 3 h (restriction site: A/CGT, http://www.neb-china.com) according to the manufacturer's directions and separated by 6% denaturing PAGE as described by Zhu et al. (2014) (Fig. S1).

Based on a SNP (BS00019095_51) on chromosome 2AL significantly associated with SD in 192 wheat varieties tested by an Illumina 90K Chip ($P < 0.001$; data not shown), a CAPS marker was developed, designated *CAPS-2AL* (F: CCCTGATGTCAAATACGGC, R: CAACTTGTAGT GCTCGGTGA), with the software Primer premier 5.0. PCR was performed in a total volume of 10 μL, including 1.0 μL of 10 × PCR buffer, 200 μM of dNTPs, 4 pmol of each primer, 0.5 U *Taq* DNA polymerase, and 100 ng of template DNA. The PCR profile included a denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, 57 °C for 30 s with a decrease of 0.1 °C per cycle, and 72 °C for 30 s and a final extension at 72 °C for 8 min. The PCR products were digested with *Nsi*I for 3 h (restriction site: ATGCA/T, http://www.neb-china.com) according to the manufacturer's directions and separated on 1.5% agarose gel (Figs. S2 and S3).

Population structure and association analysis

The population structure of the 260 wheat varieties was investigated according to the Bayesian clustering approach (Evanno et al. 2005) using the software STRUCTURE version 2.3.4 (Pritchard et al. 2000). Marker-trait associations (MTAs) were analyzed using both the general linear model (GLM) and mixed linear model (MLM) implemented in TASSEL 2.1 (Bradbury et al. 2007). For MLM analysis, marker-based kinship matrix (K) obtained using TASSEL was used along with the Q matrix to correct for both family and population structure. The significance of marker-trait association was described using P values ($P < 0.01$ for significant markers). The MTAs determined by several markers within 10 cM were assumed in one locus.

Identification of favorable alleles and their carriers

Favorable alleles of SD loci and their carriers were analyzed according to Breseghello and Mark (2006). Allele effects were estimated through comparison between the average phenotypic value of accessions with a specific allele and that of accessions with the null allele, and then the average positive or negative allele effect of the locus was calculated over the estimated phenotypic effects of all positive or negative alleles. Favorable alleles with significant effects in reducing GI were selected to identify typical carriers.

Linkage mapping and QTL analysis

Linkage mapping and QTL analysis were performed using QTL IciMapping 3.3 (Wang et al. 2013, http://www.isbreeding.net). A threshold of LOD ≥3.0 was used to construct the linkage map. Inclusive composite interval mapping (ICIM) was performed

and LOD threshold value was set using 1000 random permutations ($P < 0.05$, Churchill and Doerge 1994).

Statistical analysis

The SPSS software was used for data analysis, and correlation coefficients were estimated by the Pearson method, and *t*-tests were performed using the independent-samples *t* test (19.0 version, www.spss.com).

Results

Statistical analysis of SD data

Means across 4 years of the association mapping panel for GI5, GI15, and GI30 were 0.46, 0.60, and 0.78, respectively, ranging from 0.05 to 0.80, 0.14 to 0.90, and 0.44 to 0.96, respectively, whereas FS was 0.22 averaged over 2 years, ranging from 0.00 to 0.70. The averages of GI5-CMCC and GI15-CMCC for 201 CMCC accessions were 0.30 and 0.44, ranging from 0.02 to 0.93 and 0.01 to 0.99 in 2014 and 2015, respectively (Table S6).

Significant and positive correlations were detected among GI values at 5, 15, and 30 DAH and FS across environments in both the panels of 260 wheat varieties and 201 CMCC accessions, with correlation coefficients ranging from 0.25 to 0.95 ($P < 0.01$). A notable result was that FS showed more significant correlation with GI5 (0.43–0.61, 0.49) and GI15 (0.28–0.60, 0.43) than with GI30 (0.26–0.47, 0.33) (Tables S7 and S8).

Population structure

A set of 84 unlinked SSR markers distributed evenly on 21 wheat chromosomes was used to investigate the population structure of the 260 wheat varieties. The population could be divided into four sub-groups ($K = 4$) based on the rate of change in the log probability of data between successive K values (Fig. 1).

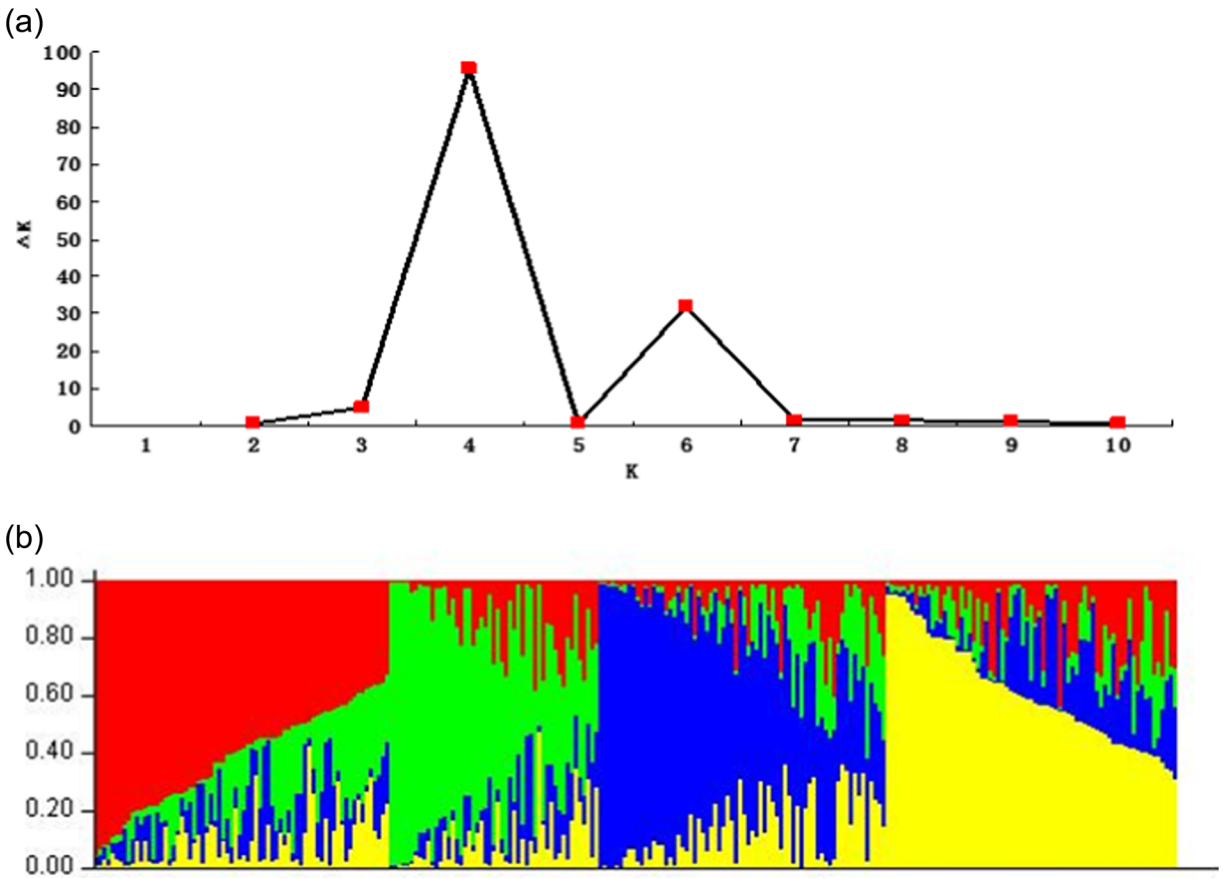

Fig. 1 Population structure of 260 wheat varieties (*lines*) based on SSR markers

Significant marker-trait associations for SD

In total, 108 MTAs at 47 loci for GI5, GI15, GI30, and FS were identified using both GLM and MLM approaches ($P < 0.01$). Among them, 72 MTAs at 41 loci were for GI5; 72 at 41 loci were for GI15; 67 at 36 loci were for GI30; and 25 at 15 loci were for FS.

Of the 47 loci, 13 were consistently associated with GI5, GI15, GI30, and FS, which were distributed on chromosomes 1AS, 1BL, 2AS, 2AL, 2BS, 3AS, 3AL, 3BL, 4BL, 5AS, 5AL, 5BL, and 7DL, respectively (Tables S9, S10, S11, S12, and S13). In particular, four loci (*TaMFT-3A* on 3AS, *Xgwm299* on 3BL, *Xbarc360* on 5AL, and *Xwmc160* on 5BL) stably associated with GI5 and GI15 in 4 years and one locus (*Xwmc658* on 2AL) consistently associated with GI5, GI15, and GI30 in 4 years, were considered major loci, explaining 4.12–17.55% of the phenotypic variation based on GLM; these were assigned to five chromosome regions, viz. END–3AS-4(0.45), 3BL-7(0.63)–END, C–5AL-12(0.35), 5BL-1(0.55)–5BL-14(0.75), and 2AL-1(0.85)–END (Table 1; Table S14).

Association analysis of 14 gene-specific markers with SD

Of 14 gene-specific markers for 7 candidate genes underlying SD/PHS resistance, 9 markers (*TaMFT-3A*, *SNP_646*, *SNP_666*, *Vp1-b2*, *Vp1B3*, *Tamyb10-A1*, *TaDFR-B*, *PM19-A1*, and *MKKAC*) showed significant association with SD at different stages of DAH, whereas the other 5 markers (*Sdr2B*, *A17–19*, *Tamyb10-B1*, *Tamyb10-D1*, and *TaMFT-A1*) were not associated with SD. *TaMFT-3A* was significantly associated with eleven SD traits across four environments, followed by *SNP_646* associated with eight SD traits in four environments and *SNP_666* associated with six SD traits in four environments; yet the other six markers were detected as associated with only one to four SD traits in one to three environments (Table 2). The result indicated *TaMFT-3A* was more closely associated with long-period seed dormancy than other genes in previous reports.

Identification of favorable alleles at major loci for SD

In total, seven, four, six, and four alleles at *Xwmc658* on 2AL, *Xgwm299* on 3BL, *Xbarc360* on 5AL, and *Xwmc160* on 5BL were present in 260 wheat varieties, respectively. Of these alleles, *Xwmc658-A154* (8, 3%),

Table 1 Major loci associated with SD at different stages after harvest in a diversity panel of 260 wheat varieties, their chromosome region, and phenotypic variation explained (PVE) by GLM in this study ($P < 0.01$)

Marker	Chromosome region	12GI5 PVE (%)	13GI5 PVE (%)	14GI5 PVE (%)	15GI5 PVE (%)	12GI15 PVE (%)	13GI15 PVE (%)	14GI15 PVE (%)	15GI15 PVE (%)	12GI30 PVE (%)	13GI30 PVE (%)	14GI30 PVE (%)	15GI30 PVE (%)	13FS PVE (%)	15FS PVE (%)
Xwmc658	2AL-1(0.85)–END	10.62	11.58	8.77	10.27	8.65	12.86	9.00	9.27	6.77	17.55	9.82	11.78		8.98
TaMFT-3A	END–3AS-4(0.45)	12.16	9.36	7.54	6.25	4.12	10.57	9.08	4.84		6.39	4.87	7.48		
Xgwm299	3BL-7(0.63)–END	8.67	9.06	7.68	5.06	8.39	8.59	8.90	6.84			6.23	5.47		
Xbarc360	C–5AL-12(0.35)	7.08	6.89	10.26	7.98	5.43	8.92	10.30	9.15	7.45		6.51			
Xwmc160	5BL-1(0.55)–5BL-14(0.75)	7.28	7.19	5.90	6.16	8.35	6.38	6.66	8.28			7.29			5.33

Table 2 Name, chromosome position, range of phenotypic variation explained (PVE) of gene-specific markers associated with SD/PHS resistance in a diversity panel of 260 wheat varieties, reported reference, and the number of traits associated in this study

Gene	Chromosome	Gene-specific marker	Range of PVE (%)	No. of trait associated	Trait associated	References
TaMFT-like	3AS	*TaMFT-3A*	4.12–12.16	11	12GI5, 12GI15, 13GI5, 13GI15, 13GI30, 14GI5, 14GI15, 14GI30, 15GI5, 15GI15, 15GI30	Nakamura et al. (2011)
		SNP_646	5.29-7.63	8	12GI5, 13GI30, 14GI5, 14GI15, 15GI5, 15GI15, 15GI30, 13FS	Liu et al. (2015)
		SNP_666	5.10-7.51	6	12GI5, 13GI30, 14GI15, 15GI5, 15GI15, 15GI30	Liu et al. (2015)
TaVp-1	3BL	*Vp1-b2*	9.78–23.40	4	12GI5, 13GI15, 13GI30, 14GI15	Chang et al. (2010a)
		Vp1B3	5.56–9.55	2	13GI15, 13GI30	Yang et al. (2007)
Tamyb10	3AL	*Tamyb10-A1*	3.79–4.62	3	13GI5, 15GI15, 14GI5	Himi et al. (2011)
TaDFR	3BL	*TaDFR-B*	4.96	1	13GI30	Bi et al. (2014)
TaPM19-A1	4AL	*PM19-A1*	4.91	1	12GI30	Barrero et al. (2015)
TaMKK3-A	4AL	*MKKAC*	5.81	1	13GI30	Torada et al. (2016)

Xwmc658-A161 (5, 2%), *Xgwm299-A200* (89, 34%), *Xbarc360-A261* (4, 2%), and *Xwmc160-A138/125* (9, 3%) had significant effects on reducing GI and FS values (Table S15). In particular, *Xwmc160-A138/125* had highest effects on reducing GI and FS values with an averaged GI5 of 0.32, GI15 of 0.31, and GI30 of 0.17 in 4 years and an averaged FS of 0.20 in 2 years, respectively. These favorable alleles showed lower distributions in Chinese varieties (2–34%). Additive effects and typical carriers of the above alleles were listed in Table S15.

Validation of the effect of favorable alleles at *Xwmc658* on SD by QTL mapping and a CAPS marker development

The PHS-tolerant varieties Wanxianbaimaizi and Suiningtuotuomai had the favorable alleles *Xwmc658-A161* and *Xwmc658-A154*, respectively. Analysis of 165 RILs from Jing 411 × Wanxianbaimaizi showed that a major QTL for 15GI5-JW, 15GI15-JW, and 15FS-JW was linked with *Xwmc658*, explaining 21.1%, 20.1%, and 9.1% of the phenotypic variations, respectively. The test of 728 F_2 plants from Jimai 20 × Suiningtuotuomai showed that a major QTL (designated *Qsd.ahau-2AL*) for 15GI5-JS and 15GI15-JS was also linked with *Xwmc658*, explaining 29.5% and 38.8% of the phenotypic variation, respectively. Additionally, a CAPS marker *CAPS-2AL* from SNP BS00019095_51 was also linked with *Xwmc658* and located at the major QTL (*Qsd.ahau-2AL*) for 15GI5-JS and 15GI15-JS on 2AL in Jimai 20 × Suiningtuotuomai population. However, there was no polymorphism between Jing 411 and Wanxianbaimaizi (Fig. 2a, b; Fig. S2; Table S16).

To further validate the association of the *CAPS-2AL* with SD, we analyzed 201 CMCC accessions representing more than 70% of the genetic diversity of Chinese wheat germplasm. A significant difference ($P < 0.01$) between the two genotypes was identified in both GI5-CMCC and GI15-CMCC across environments. The allele *CAPS-2AL-b* was significantly associated with lower GI compared with *CAPS-2AL-a*. Association analysis indicated that the *Qsd.ahau-2AL* locus explained 7.7–9.0% of the phenotypic variation in GI5-CMCC and GI15-CMCC in 2014 and 2015 (Table 3).

Discussion

Phenotypic evaluation of SD

We assayed GI at different post-ripening stages after harvest during 2011–2012, 2012–2013, 2013–2014, and 2014–2015 and FS values during 2012–2013 and 2014–2015 in 260 wheat varieties and advanced lines.

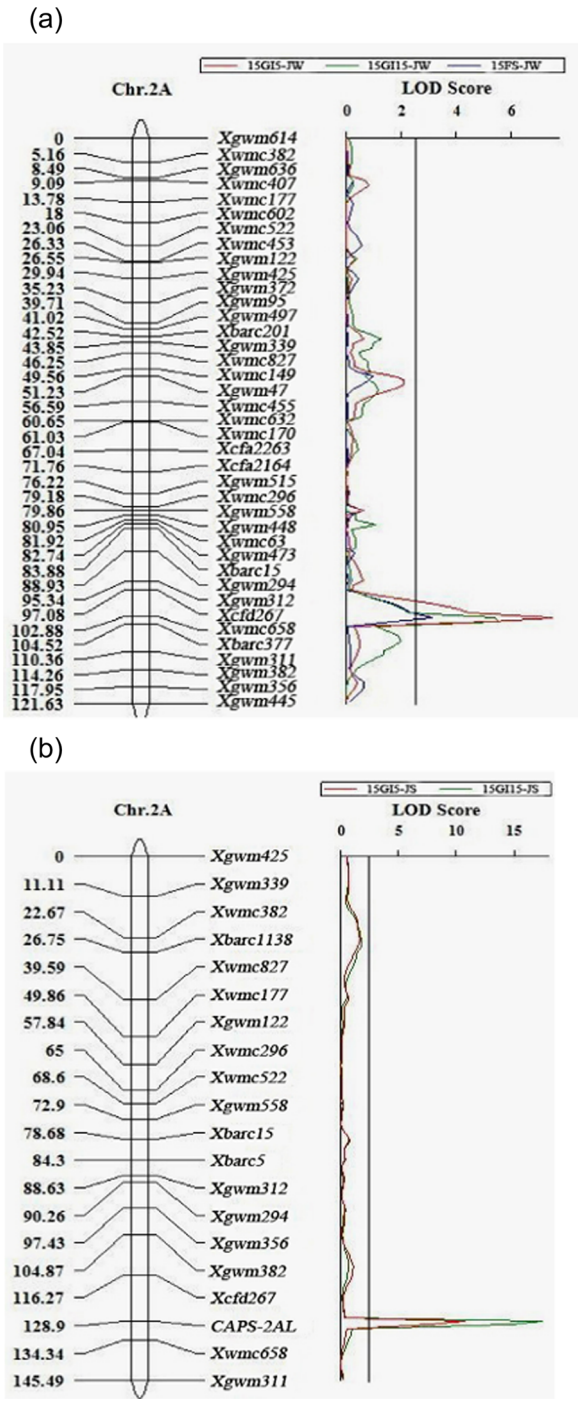

Fig. 2 Validation of the major locus near *Xwmc658* associated with SD on chromosome 2AL in Jing411 × Wanxianbaimaizi (**a**) and Jimai20 × Suiningtuotuomai (**b**) populations

The GI5, GI15, GI30, and FS values showed significant correlation across environments, confirming that seed dormancy is closely associated with intact spike sprouting which occurred in the field. Additionally, GI5 generally had more significant relationship with FS than GI15 and GI30 in different environments, suggesting that GI5 estimation is an important indicator of SD of wheat varieties. However, the breakage of seed dormancy requires a period of post-ripening, and thus, the combination analysis of GI5, GI15, GI30, and FS will be more reliable for evaluating SD duration.

Major loci for SD

For reducing the probability of false positives, both the GLM and MLM approaches were used to detect MTAs associated with SD in this study (Yu et al. 2006; Kulwal et al. 2012). In total, five major loci for SD were detected at different post-ripening stages after harvest in the present study. Particularly, the *Qsd.ahau-2AL* locus was identified to have a significant effect on maintaining longer-duration SD. Mares et al. (2002) identified a QTL located in the proximal region of chromosome 2A using a doubled haploid (DH) population derived from the cross Cranbrook × Halberd. Kumar et al. (2015) also found a QTL for GI7 on 2AL (interval *Xgwm294–Xcfa2058*) in the chromosome bins C–2AL-1(0.85) in only one environment. However, the *Qsd.ahau-2AL* locus identified in our study was near the distal end of chromosome 2AL in the region 2AL-1(0.85)–END according to Somers et al. (2004) and Sourdille et al. (2004). Therefore, we assumed that the *Qsd.ahau-2AL* locus is possibly novel. The two markers linked to *Qsd.ahau-2AL*, *Xwmc658*, and *CAPS-2AL* had a stable association with long duration of SD in different environments and genetic backgrounds of wheat. Therefore, the novel locus can be used for enhancing SD duration and PHS tolerance in wheat breeding. The result does not only provide a useful information for fine mapping and subsequent cloning of candidate genes responsible for longer-duration SD in the future but also contribute to the development of varieties with a longer period of SD in areas where prolonged rainy weather occurs frequently at harvest.

In addition, four major loci for GI5 and GI15 were also responsible for maintaining middle-duration SD. Among them, *TaMFT-3A* (Nakamura et al. 2011; Liu et al. 2013, 2015) and *Xgwm299* (Gelin et al. 2006) were confirmed to be associated with SD/PHS resistance in previous studies. Although *Xwmc160* on 5BL was not reported previously, the adjacent marker *Xgwm499* on 5BL had been identified to be associated with SD/PHS resistance in a previous study (Tan et al. 2006).

Table 3 Association of the *CAPS-2AL* allelic variations with SD in 201 Chinese wheat mini-core collections accessions

Year	Traits	Mean ± SD		*t* test	PVE (%)[a]
		CAPS-2AL-a	*CAPS-2AL-b*		
2014	14GI5-CMCC	0.36 ± 0.26	0.21 ± 0.20	4.54*	9.0
	14GI15-CMCC	0.49 ± 0.24	0.35 ± 0.23	4.36*	8.7
2015	15GI5-CMCC	0.37 ± 0.25	0.22 ± 0.19	4.57*	8.6
	15GI15-CMCC	0.52 ± 0.26	0.37 ± 0.26	4.08*	7.7

*0.01 level of *t* test of the average GI between *CAPS-2AL-a* and *CAPS-2AL-b*

[a] Percentage of phenotypic variance explained by association analysis

However, in the present study, *Xgwm499* was associated with four SD traits compared with *Xwmc160* associated with ten SD traits, indicating that the *Xwmc160* may be more closely linked to the target gene underlying SD on 5BL. Notably, the *Xbarc360* locus on 5AL and their adjacent markers identified here were also not reported previously, and it is likely a novel locus associated with SD. It is necessary to note that favorable alleles for reducing GI and FS have lower frequency distributions in Chinese wheat varieties. These alleles can be used for multi-gene pyramiding breeding for PHS resistance improvement in Chinese cultivars.

Effects of seven PHS/SD-related genes on SD

Our findings indicated that except for the *TaMFT-like* gene on chromosome 3AS (Nakamura et al. 2011; Liu et al. 2013, 2015), the *TaVp-1* (Yang et al. 2007; Chang et al. 2010a, 2011), *Tamyb10* (Himi et al. 2011), and *TaDFR* (Bi et al. 2014) on group 3 as well as *TaPM19-A1* (Barrero et al. 2015) and *TaMKK3-A* (Torada et al. 2016) on group 4 showed relatively unstable effects on SD. Moreover, *TaSdr*, an ortholog of *OsSdr4*, which is responsible for SD in wheat (Zhang et al. 2014), was not identified in this study. The reason may be due to specific genetic backgrounds of materials used. Together, our results suggest that the *TaMFT-like* gene is probably more useful for PHS resistance improvement in Chinese cultivars.

Possibility of PHS resistance genes independent of grain color in 3AL chromosome region

The relationship between grain color and PHS resistance may be due either to the pleiotropic effect of the *R* gene controlling red seed coat or to linkage between this gene and other genes underlying PHS resistance (Soper et al. 1989; Flintham 2000; Groos et al. 2002; Torada and Amano 2002; Kottearachchi et al. 2006). However, Himi et al. (2002) stated that the *R* gene may only play a minor role in grain dormancy, which tends to favor tight linkage between the *R* gene and candidate genes underlying PHS resistance. In this study, *Tamyb10*, as a strong candidate for the *R* gene, had a minor effect on SD, suggesting the presence of candidate genes underlying PHS resistance independent of the *Tamyb10* gene.

Fofana et al. (2009) identified a QTL within *Xcfa2193–Xwmc594* on 3AL for both sprouting index and seed coat color using a DH population from the cross AC Domain (red grains) × White-RL4137 (white grains). Similarly, in this study, the two markers (*Xcfa2193* and *Xwmc594*) were all significantly associated with eight SD traits, but the marker *Tamyb10-A1* for seed coat color on 3AL was only associated with three SD traits. Moreover, the above two markers and *Tamyb10-A1* were assigned to different regions, viz. C–3AL-3(0.42) (*Xcfa2193* and *Xwmc594*) and 3AL-5(0.78)–END (*Tamyb10-A1*) according to microsatellite and genetic-physical maps (Somers et al. 2004, Sourdille et al. 2004, Qi et al. 2004). Fakthongphan et al. (2016) also reported that chromosome 3A possibly carried additional loci (independent of grain color) affecting PHS resistance except for *TaPHS1* gene. These results not only provide direct evidence for possible PHS resistance genes independent of the *Tamyb10* in the 3AL chromosome region, but also verify the previous implication that the red-grained phenotype alone does not guarantee effective resistance because the marker *Tamyb10-B1* on 3BL and *Tamyb10-D1* on 3DL were not detected in this study (Flintham and Gale 1988; Kettlewell et al. 1999). Cloning of the candidate gene underlying SD/PHS resistance linked with *Tamyb10* would be beneficial for breeding varieties with PHS resistance.

Conclusion

We identified 108 MTAs at 47 loci for SD using both GLM and MLM approaches. Five major loci were significantly associated with SD at different post-ripening stages, including three loci on chromosomes 3AS, 3BL, and 5BL reported previously and two novel loci on chromosomes 2AL and 5AL. The novel locus on 2AL was further validated using three populations. A CAPS marker *CAPS-2AL* at the major locus on 2AL (designated *Qsd.ahau-2AL*) and a dCAPS marker *MKKAC* were developed to discriminate the allelic variations for *Qsd.ahau-2AL* and *TaMKK3-A*, respectively. These markers can be used for the evaluation of PHS in wheat breeding.

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Author contribution statement CC and CXM initiated the project and designed the experiment; YLZ and SXW conducted SSR and 12 gene-specific markers analysis, a CAPS and a dCAPS marker development, and prepared the manuscript; HPZ and LXZ performed linkage mapping analysis and GI and FS tests; ZYW, HJ, JJC, KL, MQ, and JL assisted in assaying all the GI and FS phenotypes; and GLS and XCX revised this paper. All authors provided comments and revisions of the manuscript.

Compliance with ethical standards

Conflict of interest We declare no conflicts of interest.

Ethical standards The experiments conducted in this study comply with the current laws of China.

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