Theor Appl Genet (2015) 128:1595–1603
DOI 10.1007/s00122-015-2534-9

ORIGINAL PAPER

Comparative fine mapping of the Wax 1 (*W1*) locus in hexaploid wheat

Ping Lu[1] · Jinxia Qin[1] · Guoxin Wang[1] · Lili Wang[1] · Zhenzhong Wang[1] · Qiuhong Wu[1] · Jingzhong Xie[1] · Yong Liang[1] · Yong Wang[1] · Deyun Zhang[1] · Qixin Sun[1] · Zhiyong Liu[1]

Received: 28 January 2015 / Accepted: 2 May 2015 / Published online: 10 May 2015

Abstract

Key message **By applying comparative genomics analyses, a high-density genetic linkage map of the Wax 1 (*W1*) locus was constructed as a framework for map-based cloning.**

Abstract Glaucousness is described as the scattering effect of visible light from wax deposited on the cuticle of plant aerial organs. In wheat, the wax on leaves and stems is mainly controlled by two sets of genes: glaucousness loci (*W1* and *W2*) and non-glaucousness loci (*Iw1* and *Iw2*). Bulked segregant analysis (BSA) and simple sequence repeat (SSR) mapping showed that Wax1 (*W1*) is located on chromosome arm 2BS between markers *Xgwm210* and *Xbarc35*. By applying comparative genomics analyses, colinearity genomic regions of the *W1* locus on wheat 2BS were identified in *Brachypodium distachyon* chromosome 5, rice chromosome 4 and sorghum chromosome 6, respectively. Four STS markers were developed using the *Triticum aestivum* cv. Chinese Spring 454 contig sequences and the International Wheat Genome Sequencing Consortium (IWGSC) survey sequences. *W1* was mapped into a 0.93 cM genetic interval flanked by markers *XWGGC*3197 and *XWGGC*2484, which has synteny with genomic regions of 56.5 kb in *Brachypodium*, 390 kb in rice and 31.8 kb in sorghum. The fine genetic map can serve as a framework for chromosome landing, physical mapping and map-based cloning of the *W1* in wheat.

Communicated by I. D. Godwin.

✉ Zhiyong Liu
zhiyongliu@cau.edu.cn

[1] State Key Laboratory for Agrobiotechnology/Department of Plant Genetics and Breeding, China Agricultural University, Beijing 100193, China

Introduction

The wheat leaf, stem and, in some cases, spike surfaces are coated with cuticular waxes, which confers a glaucousness characteristic (Jensen and Driscoll 1962). Epicuticular waxes are composed of several classes of compounds, including very-long-chain fatty acids (VLCFA; >18C), esters, primary and secondary alcohols, fatty aldehydes and ketones (Kunst and Samuels 2003). As a barrier between plants and their environment, the outermost wax layer functions in defending plants against the biotic and abiotic stresses, such as drought, phytophagous insects, pathogens, solar radiation, and freezing temperatures (Eigenbrode and Espelie 1995; Jenks and Ashworth 1999). Worldwide, bread wheat (*Triticum aestivum* L.) is one of the most important food sources for human beings. However, drought has long been a major threat to global crop production, and climate change exaggerates its scale and frequency. One of the most important functions of the cuticular wax is that it restricts non-stomatal water loss and protects plants against ultraviolet radiation and reduces water retention on the plant surface, thus minimizing deposition of dust, pollen and air pollutions (Kunst and Samuels 2003). Moreover, glaucousness significantly increased grain and biomass yield in irrigated and rain-fed field experiments in wheat (Johnson et al. 1983). Recently, Zhang et al. (2013) demonstrated that glaucousness reduced cuticle permeability in the terms of non-stomatal water loss and chlorophyll efflux.

Genetic analyses have revealed that the glaucousness and non-glaucousness phenotypes on wheat stem and leaf are mainly controlled by two sets of loci: the wax production genes *W1* and *W2* and the wax inhibitor genes *Iw1* and *Iw2*, respectively (Jensen and Driscoll 1962; Tsunewaki 1966; Tsunewaki and Ebana 1999). The wax production gene *W1* and the wax inhibition gene *Iw1* are closely linked

on chromosome 2BS (Tsunewaki 1966). However, the wax inhibition gene *Iw2* is located on the distal of 2DS where the wax production gene *W2* is close to the centromere (Tsunewaki and Ebana 1999). Another two loci, *Iw3* and *Ws*, both derived from *T. dicoccoides*, were reported on 1BS (Dubcovsky et al. 1997) and 1AS (Gadaleta et al. 2009) conditioning wax on spikes in wheat. Recently, QTL for flag leaf glaucousness was also detected in wheat (Bennett et al. 2012).

High-resolution genetic linkage maps for wax inhibition genes *Iw1* (Adamski et al. 2013; Wu et al. 2013), *Iw2* (Wu et al. 2013) and *Iw3* (Wang et al. 2014a) have been constructed. However, very little information is available for the wax production genes. (Tsunewaki 1966) demonstrated that the wax production gene *W1* is located 2 cM away from the wax inhibition gene *Iw1* on 2BS. Recent study suggested that the *W1* gene was tightly linked with molecular markers *Xbarc35* and *Xwmc764* on chromosome 2BS (Yoshiya et al. 2011).

Fine mapping and map-based cloning in common wheat are difficult because of the large wheat genome size (17 Gb), polyploidy (AABBDD), and highly repetitive DNA (90 %). The availability of wheat expressed sequence tags (EST) and the rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* L.), and *Brachypodium distachyon* genome sequences provide comparative genomics tools for wheat gene mapping and map-based cloning. The high-density genetic linkage maps of vernalization (*VRN*) genes (Yan et al. 2003, 2004, 2006), pairing homologous 1 (*Ph1*) (Griffiths et al. 2006), grain protein content-B1 (*Gpc-B1*) (Uauy et al. 2006), yellow rust resistance gene *Yr36* (Fu et al. 2009) and powdery mildew resistance gene *MlIW172* (Ouyang et al. 2014) were constructed through comparative genomics analysis. The recently released draft genome sequences of *T. aestivum* cv. Chinese Spring, *T. urartu* accession G1812 and *Aegilops tauschii* accession AL8/78 provide nearly complete gene sets of the wheat A, B and D genomes (Brenchley et al. 2012; Jia et al. 2013; Ling et al. 2013) for marker development and gene identification.

In this paper, we report the comparative genomic analysis and high-resolution genetic linkage map construction of the wax production gene Wax 1 (*W1*) as a framework for map-based cloning and marker-assisted selection in wheat breeding programs.

Materials and methods

Plant materials

Common wheat lines P86 (glaucousness) and J87 (non-glaucousness) are derivatives of two most popular commercialized common wheat cultivars Jimai20 and Aikang58 in North China. A 4508 plant F_2 population developed from the cross between P86 (Fig. 1a) and J87 (Fig. 1b) was used for genetic analysis and fine mapping. The parental lines, F_1 (Fig. 1c), F_2 segregating population and F_3 families were grown in field trails under well-watered condition at Beijing, China. Each F_2 plant was bagged to harvest seeds for F_3 family phenotyping. The glaucousness phenotype was visualized and recorded after flowering at Feekes' stage 10.5.1 (Large 1954) when the glaucousness was fully expressed in the leaf, stem and spike (Fig. 1).

Chinese Spring (CS) and its nullisomic–tetrasomics, ditelosomics and deletion lines of homoeologous group 2 (kindly provided by Drs. WJ Raupp and BS Gill, Wheat Genetics Resource Centre, Kansas State University, USA) were used for chromosomal arm assignment and bin mapping of molecular markers flanking the wax production genes *W1* in P86.

Fig. 1 Visual differences arising from the presence of different alleles of *W1* locus in the field (**a**, **b**), the F_1 plants in the field (**c**), and single tillers (**d**)

Polymerase chain reaction (PCR) and molecular marker analysis

Total genomic DNA was isolated from leaves by use of a cetyltrimethylammonium bromide (CTAB) protocol (Saghai-Maroof et al. 1984). For bulked segregant analysis, glaucous and non-glaucous bulks were established by mixing equal amounts of genomic DNA from ten homozygous glaucous and ten homozygous non-glaucous F_2 plants from the segregating population. Wheat genomic SSRs (*Xgwm*, *Xwmc*, *Xbarc*, *Xcfa*, and *Xcfd* series) and EST markers were chosen for polymorphism analyses. Primer sequences information of these genomic SSR and EST markers is available online at GrainGenes website (http://wheat.pw.usda.gov). The resulting polymorphic markers were used to genotype the F_2 populations.

Polymerase chain reaction (PCR) was performed in 10 µl reactions containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 25 ng of each primer, 50 ng of genomic DNA, and 0.75U of TaqDNA polymerase. DNA amplification was performed at 94 °C for 5 min, followed by 30 cycles at 94 °C for 45 s, 50–60 °C (depending on specific primers) for 45 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were mixed with 2 µl of loading buffer (98 % formamide, 10 mM EDTA, 0.25 % bromophenol blue, and 0.25 % xylene cyanol), separated on 8 % non-denaturing polyacrylamide gels (39 acrylamide: 1 bisacrylamide), and visualized following silver staining.

Comparative genomics analysis and EST marker development

To locate genomic regions homologous to the wheat wax production locus *W1* in *Brachypodium*, rice and sorghum genomes, EST sequences flanking the *W1* locus were used to perform BLAST searches of *Brachypodium* (http://mips.helmholtz-muenchen.de/plant/brachypodium/), rice (http://rice.plantbiology.msu.edu/) and sorghum (http://mips.helmholtz-muenchen.de/plant/sorghum/) genome sequence databases with an e-value cutoff of 10e-10. Then, orthologous gene pairs between *Brachypodium*, rice and sorghum were compared within the homologous genomics regions. The putative gene pairs with high level of colinearity among *Brachypodium*, rice, and sorghum were preferentially used to search homologous wheat ESTs (http://www.ncbi.nlm.nih.gov/)to develop new polymorphic markers linked to the *W1* locus.

After verifying the orthologous genomic regions, the coding sequences (CDS) of orthologous gene pairs among *Brachypodium*, rice, and sorghum were used as queries to search the NCBI database (http://www.ncbi.nlm.nih.gov/) for orthologous wheat ESTs, which were subsequently used to search the *T. aestivum* cv. Chinese Spring 454 contig sequences (Brenchley et al. 2012) and the International Wheat Genome Sequencing Consortium (IWGSC) Chinese Spring chromosome 2BS survey sequences (http://www.wheatgenome.org/). The orthologous Chinese Spring contigs were used as template to design primers with Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/). The primer designing parameters were as follows: amplification product size of 150–600 bp, primer length of 18–25 bp with the optimum 20 bp, T_m of 55–65 °C with the optimum 60 °C, and GC content of 40–60 %. Adjacent amplicons overlapped to ensure amplification of the entire contig. The designed primers were screened for polymorphisms between the parental lines, as well as the glaucous and non-glaucous DNA bulks. The chromosome 2BS-specific polymorphic STS markers were used for F_2 genotyping to construct the high-density genetic linkage map.

High-density genetic linkage map construction

Chi-squared (χ^2) tests for goodness-of-fit were used to evaluate deviations of observed data from theoretically expected segregation ratios. Linkages between markers and the *W1* gene were determined using Mapmaker 3.0, with a LOD score threshold of 3.0 (Lincoln et al. 1992). The genetic map was constructed with the software Mapdraw V2.1 (Liu and Meng 2003).

Results

Genetic analysis of wax production gene *W1* in hexaploid wheat

At the adult plant stage, leaves and stems of the common wheat line P86 are glaucous, whereas for the line J87 are non-glaucous. The F_1 plants from the cross of J87/P86 are glaucous (Fig. 1c), suggesting that the wax production gene in P86 is dominant. The F_2 population of J87/P86 segregated as 3384 glaucous and 1124 non-glaucous, which fits a 3:1 ratio ($p = 0.918$). The $F_{2:3}$ progenies segregated as 1134 homozygous glaucous: 2250 segregating: 1124 homozygous non-glaucous, as expected for a single gene segregation ratio of 1:2:1($p = 0.971$). This result suggests that glaucous in P86 is controlled by a single dominant wax production gene.

Identification and physical bin mapping of polymorphic markers linked to *W1*

First, 224 SSR primers (*Xgwm, Xwmc*, *Xbarc*, *Xcfa*, and *Xcfd* series) distributed randomly throughout the whole genome were surveyed for polymorphisms between the

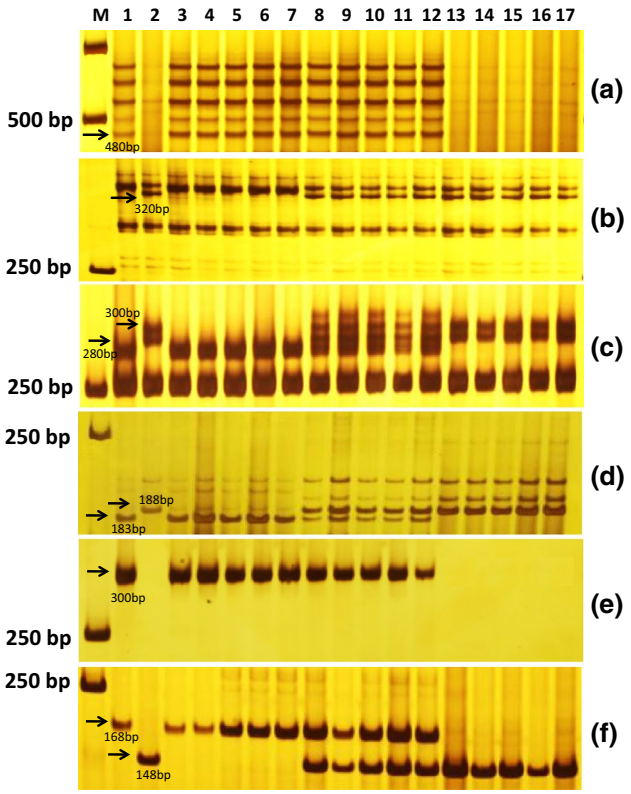

Fig. 2 PCR amplification patterns of polymorphisms markers, *Xgwm210* (**a**), *Xbarc35* (**b**), *XWGGC2213* (**c**), *XWGGC2323* (**d**), *XWGGC2484* (**e**), and *XWGGC3197* (**f**). *Lanes 1* and *2* are parental lines P86 and J87, respectively. *Lanes 3–7* homozygous glaucous F_2 plants, *lanes 8–12* heterozygous glaucous F_2 plants and *lanes 13–17* homozygous non-glaucous F_2 plants

parental lines and between glaucous and non-glaucous DNA bulks. Two SSR markers, *Xgwm210* and *Xbarc35* (Fig. 2a, b), detected such polymorphisms and the wax locus was mapped between them.

Since *Xgwm210* was mapped on chromosome 2AS, 2BS, and 2DS and *Xbarc35* was mapped only on 2BS (Somers et al. 2004), Chinese Spring homoeologous group 2 nullisomic–tetrasomics, ditelosomics, and deletion lines were used to assign the chromosomal and physical bin locations of the wax production locus and its linked SSR markers. *Xbarc35* was not detected in N2B-T2D, Dt2BL, deletion lines 2BS-1, 2BS-4 and 2BS-3, but present in N2A-T2B, N2D-T2A (Fig. 3), indicating that *Xbarc35* is located on chromosome 2BS bin 0.84–1.00. Moreover, *Xgwm210* was located on chromosome 2BS bin 0.84–1.00 (Somers et al. 2004). Both polymorphic bands of the SSR markers listed above could be mapped on the 2BS bin 0.84–1.00 (Fig. 4a), indicating that the wax locus was also located in that bin and should be the wax production gene Wax 1 (*W1*) (Fig. 4c).

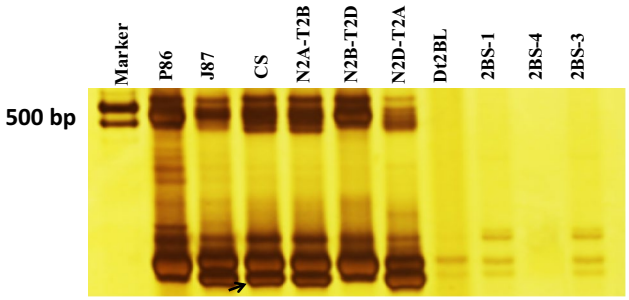

Fig. 3 Amplification patterns of markers *Xbarc35* in the parental lines P86 and J87, Chinese Spring (CS) and its homoeologous group 2 nullisomic–tetrasomics, ditelosomics, and deletion lines

Then, ESTs bin-mapped on chromosome 2BS bin 0.84–1.00 were tested for polymorphisms between the parental lines as well as the glaucous and non-glaucous DNA bulks. Out of 86 EST-STS primer pairs tested, two EST-derived STS markers, *XWGGC2213*(developed from CD927782) and *XWGGC2323* (developed from CJ722335) (Fig. 2c, d), were found to be polymorphic between the glaucous and non-glaucous DNA bulks and linked to the *W1* locus at 0.44 cM proximal and 0.88 cM distal, respectively (Fig. 4c).

Comparative genomics analysis

To saturate the *W1* genetic map, the sequences of CD927782 and CJ722335 flanking *W1* were used as queries to search the rice, sorghum and *Brachypodium* genome sequences to identify syntenic genomic regions corresponding to the *W1* locus in wheat. CJ722335 detected putative orthologs on *Brachypodium* chromosome 5 (Bd5g01220), rice chromosome 4 (Os04g0132300) and sorghum chromosome 6 (Sb06g001290). A putative ortholog of CD927782 was found in rice (Os04g0136900), but not in *Brachypodium* and sorghum. Detailed comparison indicated that a 134 kb genomic region in the *Brachypodium* chromosome 5S from Bd5g01130 to Bd5g01230 is syntenic to a 487 kb region in rice chromosome 4S from Os04g0137100 to Os04g0131900, and a 227 kb region in sorghum chromosome 6S from Sb06g001410 to Sb06g001270 (Table 1; Fig. 4d, e, f). Comparative analyses revealed that 6 out of the 11 predicted *Brachypodium* genes in this region (Bd5g01130, Bd5g01140, Bd5g01180, Bd5g01210, Bd5g01220 and Bd5g01230) have orthologous genes in rice and sorghum. The *Brachypodium* genes Bd5g01130 and Bd5g01140 correspond to one gene in rice (Os04g0137100) and one gene in sorghum (Sb06g001410), indicating gene duplications in *Brachypodium*. However, genomic rearrangements are observed in the syntenic genomic regions between wheat, *Brachypodium*, rice and

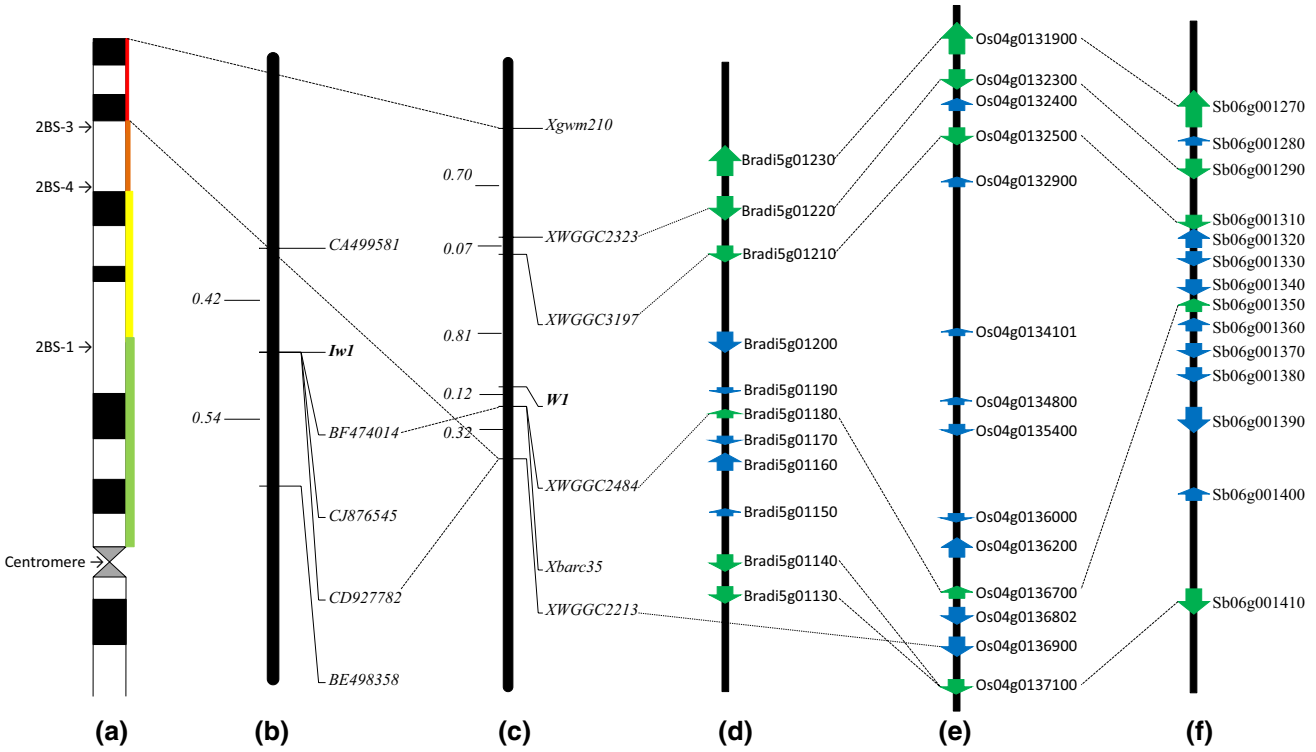

Fig. 4 Comparative high-resolution genetic linkage maps of the Wax 1 (*W1*) locus in wheat. **a** *W1* was mapped to distal bin 2BS bin 0.84–1.00. **b** Genetic linkage map of *Iw1* on wheat chromosome 2BS (Wu et al. 2013). **c** Genetic linkage map of *W1* on wheat chromosome 2BS with genetic distances in cM shown on the *left*, markers shown on the *right*. **d** The orthologous genomic region of *W1* on *Brachypodium* chromosome 5 with putative genes on the *right*. **e** The orthologous genomic region of *W1* on rice chromosome 4 with putative genes on the *right*. **f** The orthologous genomic region of *W1* on sorghum chromosome 6 with putative genes on the *right*. *Arrows* the transcriptional direction of orthologous genes

sorghum (Table 1; Fig. 4d, e, f). Detailed annotation and comparative analysis revealed that no orthologs of 5 *Brachypodium* genes, Bd5g01150, Bd5g01160, Bd5g01170, Bd5g01190 and Bd5g01200 presented in the rice and sorghum orthologous genomic regions.

Polymorphic markers development and fine mapping

The putative orthologous gene pairs of *Brachypodium*, rice and sorghum were preferentially used to search wheat homolog ESTs or Chinese Spring contigs to develop molecular markers linked to *W1*. Two polymorphic marker *XWGGC2484* and *XWGGC3197* (Table 2; Fig. 2e, f) were developed using the coding sequence (CDS) of *Brachypodium* genes Bd5g01180 and Bd5g01210 as queries and genotyped in the F_2 population to narrow the genetic region of the wax production gene *W1*. Finally, *W1* was mapped between *XWGGC2484* and *XWGGC3197* in a genetic interval of 0.93 cM, which is colinear with genomic regions of 56.5 kb (Bd5g01180-Bd5g01210) in *Brachypodium*, 390 kb (Os04g0136700-Os04g0132500) in rice, and 31.8 kb (Sb06g001350-Sb06g001310) in sorghum (Fig. 4).

Discussion

Plant cuticles provide the first line of defense between plant and its environment. The cuticle is covered by epicuticular waxes whose chemical and physical properties have important roles in resistance to biotic and abiotic stress. In wheat and its relatives, almost all species have parallel variations of glaucous and non-glaucous, except for Einkorn (A genome) which is non-glaucous (Bianchi and Figini 1986; Tsunewaki 1966). Two dominant genes, Wax 1 (*W1*) and Wax 2 (*W2*), for glaucous were reported and cytologically located on chromosomes 2BS and 2DS, respectively (Tsunewaki 1966, 1962). Genetic study also revealed that *W1* is 2.0 cM proximal to wax inhibition gene *Iw1* on distal of 2BS (Tsunewaki 1966). Recent molecular mapping study indicated that the epicuticular wax gene *W1* was tightly linked with SSR markers *Xbarc35* and *Xwmc764* on chromosome 2BS (Yoshiya et al. 2011). In a fine mapping population, the wax inhibition gene *Iw1* was found to be co-segregated with markers CD927782 and BF474014 (Fig. 4b; Wu et al. 2013). Our fine mapping results demonstrated that both EST-STS makers *XWGGC2213* (developed from CD927782) and *XWGGC2484* (developed from

Table 1 Colinearity between *Brachypodium*, rice and sorghum in the syntenic genomic region of wheat Wax 1 (*W1*) locus

Wheat EST	*Brachypodium*	*E* value	Identity[a]	Rice	Sorghum	Annotation	Chinese spring contigs location
CJ777783	Bd5g01230	0	90 %	Os04g0131900	Sb06g001270	UDP-glucose: sterol glucosyltransferase	2BS
					Sb06g001280	Succinate dehydrogenase subunit 3	2BS
CJ722335	Bd5g01220	0	88 %	Os04g0132300	Sb06g001290	AAR2 family protein	2BS
				Os04g0132400		Conserved hypothetical protein	2BS
AL819694	Bd5g01210	e−103	89 %	Os04g0132500	Sb06g001310	LRR receptor-like serine/threonine-protein	2BS
	Bd5g01200					*N*-acylethanolamine amidohydrolase	2BS
	Bd5g01190					Unknown protein/serine-type peptidase	2BS
				Os04g0132900		Conserved hypothetical protein	–
				Os04g0134101		Non-protein coding transcript	7AL
				Os04g0134800		Haem peroxidase, plant/fungal/bacterial family protein	1BS
				Os04g0135400		Protein kinase domain containing protein	2AS
				Os04g0136000		Protein kinase domain containing protein	2BS
				Os04g0136200		Copper methylamine oxidase precursor	2BL
					Sb06g001320	Hydrolase/zinc ion binding protein	2BS
					Sb06g001330	F-box domain containing protein	2BL
					Sb06g001340	Histone-lysine *N*-methyltransferase	4DL
BF474014	Bd5g01180	e−122	92 %	Os04g0136700	Sb06g001350	CBS domain containing protein	2BS
	Bd5g01170					NB-ARC domain containing protein	2BS
CJ876545	Bd5g01160	e−139	90 %			LIM, zinc binding; Ubiquitin interacting motif	2BS
	Bd5g01150					Plant lipid transfer protein	2BS
				Os04g0136802		Conserved hypothetical protein	–
CD927782		8e−35	86 %	Os04g0136900		Cell number division protein	2BS
					Sb06g001360	DNA-directed RNA polymerase II subunit RPB11	5AL
					Sb06g001370	D-isomer specific 2-hydroxyacid dehydrogenase-like protein	2BS
					Sb06g001380	Serine-rich 25 kDa antigen protein	2BS
					Sb06g001390	Lysine ketoglutarate reductase trans-splicing related 1	4BS
					Sb06g001400	Conserved hypothetical protein	3AS
BE498358	Bd5g01140	9e−44	90 %	Os04g0137100	Sb06g001410	Probable pectate lyase 15-like	2BS
	Bd5g01130	9e−44	90 %				

[a] Nucleotide identity between wheat EST and orthologous *Brachypodium* gene (rice for CD927782)

BF474014) were proximal to the *W1* locus with genetic distance of 0.44 and 0.12 cM, respectively, indicating that the *W1* locus is distal to the *Iw1* locus on 2BS.

Comparative genomics analyses have been extensively exploited in wheat to develop high-resolution genetic linkage maps and clone genes of interest (Guyot et al. 2004; Schnurbusch et al. 2007). This approach is proved increasingly powerful due to the generation of genome sequences from closely related species including *B. distachyon*, rice, sorghum, and maize. Moreover, the recently released shotgun genome sequences of hexaploid wheat cv. Chinese Spring (Brenchley et al. 2012) and the wheat chromosome

Table 2 EST-STS markers tightly linked to the Wax 1 (*W1*) locus

Markers	Wheat ESTs	Forward primer (5′–3′)	Reverse primer (5′–3′)
XWGGC2323	CJ722335	TGAAGCGTGGAACACACAT	GCTCAACACATAAGGCATCA
XWGGC3197	AL819694	CGCAGTTATGACAGAGATCA	GTACATTCTCCACACATCCAC
XWGGC2484	BF474014	GCTCTCGCTCACCTTCTAT	CGTAGCATCTATCTAAACATCG
XWGGC2213	CD927782	TCAGGCAACCAAAACCCTTA	CCTTTTCTCCAGCTCAATCG

arm survey assemblies (Mayer et al. 2014; http://www.wheatgenome.org/) provide key resources for wheat marker development. By applying comparative genomic analyses, the wax inhibition gene *Iw1* (Adamski et al. 2013; Wu et al. 2013) and powdery mildew resistance genes *MlIW172* and *Pm41* (Ouyang et al. 2014; Wang et al. 2014b) have been narrowed to sub-centimorgan intervals in wheat. Using a similar strategy, we mapped the wax gene *W1* into a 0.93 cM interval between markers *XWGGC3197* and *XWGGC2484* that corresponds to 56.5, 390 and 31.8 kb orthologous genomic regions in *Brachypodium* chromosome 5S, rice chromosome 4S and sorghum 6S, respectively (Fig. 4).

Macro-colinearity has been observed between wheat homoeologous group 2 chromosomes and *Brachypodium* chromosome 5, rice chromosome 4, sorghum chromosome 6 and barley chromosome 2 (Brenchley et al. 2012). The synteny relationship was conformed between the *W1* genetic region on 2BS and the short arms of *Brachypodium* chromosome 5, rice chromosome 4 and sorghum chromosome 6. However, genomic rearrangements were observed between the wheat *W1* genetic region and orthologous genomic regions of *Brachypodium*, rice and sorghum. Among the 11, 10 and 13 predicted genes in the *Brachypodium*, rice and sorghum orthologous genomic regions, 5 genes are orthologs across the three species (Table 1; Fig. 4). Bd5g01130 and Bd5g01140 are duplicated genes therefore being considered as one gene. In other word, 5, 5, and 8 predicted genes are species-specific genes for *Brachypodium*, rice and sorghum, respectively, within this region. Marker *XWGGC2213*, developed from the non-syntenic rice gene Os04g0136900, was located 0.32 cM proximal to marker *XWGGC2482*, developed from syntenic *Brachypodium* gene Bd5g01180, implying that non-syntenic rice gene can also provide alternative information for marker development when mapping wheat genes.

Further attempts to narrow the genetic interval of *W1* between *XWGGC3197* and *XWGGC2484* were not successful when the non-syntenic genes Bd5g01190 and Bd5g01200 from *Brachypodium*, Os04g0132900, Os04g0134101, Os04g0134800, Os04g0135400, Os04g0136000 and Os04g0136200 from rice, Sb06g001320, Sb06g001330, and Sb06g001340 from sorghum were used as queues to Blast wheat Contigs for marker development. The *Brachypodium* genes Bd5g01190 and Bd5g01200, the rice genes Os04g0132900, Os04g0134101 and Os04g0136000, the sorghum gene Sb06g001330 have no orthologous genes in the corresponding genomic regions among the three species. Homologous genes of Os04g0134800 and Os04g0136200 are identified at non-orthologous chromosomes as Bd2g37060 and Bd1g50950 in *Brachypodium,* Sb04g020720 and Sb06g020020 in sorghum, respectively. The ortholog of Os04g0135400 is Bradi4g40470 in *Brachypodium.* Both Sb06g001320 and Sb06g001340 have orthologous genes Bradi1g50950 and Bradi1g53840 in *Brachypodium,* Os09g0128600 and Os11g0602200 in rice, respectively.

Although these genes have orthologous genes in *Brachypodium*, rice and sorghum genomes, however, they are not located in the syntenic genomics regions therefore could not be used for marker development in wheat. The homologs of some of these non-orthologous genes were not located on chromosome 2BS but other chromosome arms in the Chinese Spring chromosome sorting survey sequences (Table 1; Mayer et al. 2014). These non-syntenic genes might be the results of genome rearrangements after the speciational divergence of the four species. Since no even closely or co-segregated markers for *W1* can be developed to fill the gaps between *XWGGC3197* and *XWGGC2482*, the EST-STS markers *XWGGC2484* and *XWGGC3197* can then be served as starting points for chromosome landing, physical mapping and map-based cloning of the glaucousness gene *W1* in wheat.

Numerous wheat genes for agronomic traits and disease resistance have been identified on chromosome 2BS. The photoperiod insensitive gene *Ppd-B1* and additional genetic factor for influencing ear emergence time have been known to be related with chromosome 2BS of wheat (Mohler et al. 2004; Scarth and Law 1983). The *Ppd-B1* was mapped 34.1 cM proximal to SSR marker *Xgwm210* (Mohler et al. 2004). However, the Wax1 (*W1*) locus is tightly linked to *Xgwm210* at a genetic distance of 1.58 cM, indicating the *W1* is not closely linked to *Ppd-B1*. Furthermore, no significant correlation between the glaucousness and the heading date was observed in our mapping population at field condition.

Several publications revealed that the glaucousness in wheat leaf, stem and spike surface reduces transpiration and increases water use efficiency (WUE) under drought or stressed condition (Johnson et al. 1983; Richards et al.

1986). However, under well-watered condition in this research, the average thousand grains weights (TGW) of 80 homozygous glaucous F_2 plants and 76 homozygous non-glaucous F_2 plants are 39.6 gram and 39.5 gram, respectively, indicating no significant difference between the glaucous and non-glaucous phenotypes on TGW. Further study using recombinant inbred lines (RIL) or near isogenic lines (NIL) should be tested in future on water-limited or stressed condition to characterize the influence of epicuticular wax on yield, WUE and stress tolerance.

Moreover, many disease resistance genes, such as *Pm26* (Rong et al. 2000), *Pm42* (Hua et al. 2009), *Lr16* (McCartney et al. 2005), *Yr31* (Singh et al. 2003) and so on were located on 2BS. As a result, the chromosome 2BS is rich in germplasm and genomic resources. These resources and molecular markers developed in this research provide opportunity for molecular manipulation of the agronomic traits and disease resistance genes via marker-assisted selection in wheat breeding program.

Author contribution statement PL, JQ and ZL designed the experiments; PL, JQ, GW, LW, ZW, QW, JX, YL, YW, DZ performed the experiments; QS, JQ, ZL provided materials; PL and ZL wrote the paper.

Acknowledgments This work was financially supported by the Ministry of Science and Technology of China (2011AA100104, 2011CB100104), National Science Foundation of China (31030056, 31210103902) and Ministry of Education (MOE) of China (111-02-3).

Conflict of interest The authors have declared that no conflict of interest.

References

Adamski NM, Bush MS, Simmonds J, Turner AS, Mugford SG, Jones A, Findlay K, Pedentchouk N, von Wettstein-Knowles P, Uauy C (2013) The Inhibitor of wax 1 locus (*Iw1*) prevents formation of—and OH-β-diketones in wheat cuticular waxes and maps to a sub-cM interval on chromosome arm 2BS. Plant J 74:989–1002

Bennett D, Izanloo A, Edwards J, Kuchel H, Chalmers K, Tester M, Reynolds M, Schnurbusch T, Langridge P (2012) Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions. Theor Appl Genet 124:697–711

Bianchi G, Figini ML (1986) Epicuticular waxes of glaucous and nonglaucous durum-wheat lines. J Agric Food Chem 34:429–433

Brenchley R, Spannagl M, Pfeifer M, Barker GL, D'Amore R, Allen AM, McKenzie N, Kramer M, Kerhornou A, Bolser D (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491:705–710

Dubcovsky J, Echaide M, Giancola S, Rousset M, Luo MC, Joppa LR, Dvorak J (1997) Seed-storage-protein loci in RFLP maps of diploid, tetraploid, and hexaploid wheat. Theor Appl Genet 95:1169–1180

Eigenbrode SD, Espelie KE (1995) Effects of plant epicuticular lipids on insect herbivores. Ann Rev Entomol 40:171–194

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

Gadaleta A, Giancaspro A, Giove SL, Zacheo S, Mangini G, Simeone R, Signorile A, Blanco A (2009) Genetic and physical mapping of new EST-derived SSRs on the A and B genome chromosomes of wheat. Theor Appl Genet 118:1015–1025

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G (2006) Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. Nature 439:749–752

Guyot R, Yahiaoui N, Feuillet C, Keller B (2004) In silico comparative analysis reveals a mosaic conservation of genes within a novel colinear region in wheat chromosome 1AS and rice chromosome 5S. Funct Integr Genomics 4:47–58

Hua W, Liu ZJ, Zhu J, Xie CJ, Yang TM, Zhou YL, Duan XY, Sun QX, Liu ZY (2009) Identification and genetic mapping of *pm42*, a new recessive wheat powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. *dicoccoides*). Theor Appl Genet 119:223–230

Jenks MA, Ashworth EN (1999) Plant epicuticular waxes: function, production, and genetics. Hortic Rev 23:1–68

Jensen NF, Driscoll CJ (1962) Inheritance of the waxless character in wheat. Crop Sci 2:504–505

Jia JZ, Zhao SC, Kong XY, Li YR, Zhao GY, He WM, Appels R, Pfeifer M, Tao Y, Zhang XY, Jing RL, Zhang C, Ma YZ, Gao LF, Gao C, Spannagl M, Mayer KFX, Li D, Pan SK, Zheng FY, Hu Q, Xia XC, Li JW, Liang QS, Chen J, Wicker T, Gou CY, Kuang HH, He GY, Luo YD, Keller B, Xia QJ, Lu P, Wang JY, Zou HF, Zhang RZ, Xu JY, Gao JL, Middleton C, Quan ZW, Liu GM, Wang J, Yang HM, Liu X, He ZH, Mao L, Wang J (2013) *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation. Nature 496:91–95

Johnson DA, Richards RA, Turner NC (1983) Yield, water relations, gas exchange, and surface reflectances of near-isogenic wheat lines differing in glaucousness. Crop Sci 23:318–325

Kunst L, Samuels AL (2003) Biosynthesis and secretion of plant cuticular wax. Prog Lipid Res 42:51–80

Large EC (1954) Growth stages in cereals, illustration of the Feekes Scales. Plant Path. 3:128–129

Lincoln S, Daly M, Lander E (1992) Constructing genetic maps with Mapmaker/eXP3.0 Whitehead Institute Techn rep, 3rd edn. Whitehead Institute, Cambridge

Ling HQ, Zhao SC, Liu DC, Wang JY, Sun H, Zhang C, Fan HJ, Li D, Dong LL, Tao Y, Gao C, Wu HL, Li YW, Cui Y, Guo XS, Zheng SS, Wang B, Yu K, Liang QS, Yang WL, Lou XY, Chen J, Feng MJ, Jian JB, Zhang XF, Luo GB, Jiang Y, Liu JJ, Wang ZB, Sha YH, Zhang BR, Wu HJ, Tang DZ, Shen QH, Xue PY, Zou SH, Wang XJ, Liu X, Wang FM, Yang YP, An XL, Dong ZY, Zhang KP, Zhang XQ, Luo MC, Dvorak J, Tong YP, Wang J, Yang HM, Li ZS, Wang DW, Zhang AM, Wang J (2013) Draft genome of the wheat A-genome progenitor *Triticum urartu*. Nature 496:87–90

Liu R, Meng J (2003) MapDraw: a microsoft excel macro for drawing genetic linkage maps based on given genetic linkage data. Hereditas (Beijing) 25:317–321

Mayer KF, Rogers J, Doležel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P, Endo TR, Kubaláková M, Cíhalíková J, Dubská Z, Vrána J, Sperková R, Simková H, Febrer M, Clissold L, McLay K, Singh K, Chhuneja P, Singh NK, Khurana J, Akhunov E, Choulet F, Alberti A, Barbe V, Wincker P, Kanamori H, Kobayashi F, Itoh T, Matsumoto T, Sakai H, Tanaka T, Wu J, Ogihara Y, Handa H, Maclachlan PR,

Sharpe A, Klassen D, Edwards D, Batley J, Olsen OA, Sandve SR, Lien S, Steuernagel B, Wulff B, Caccamo M, Ayling S, Ramirez-Gonzalez RH, Clavijo BJ, Wright J, Pfeifer M, Spannagl M, Martis MM, Mascher M, Chapman J, Poland JA, Scholz U, Barry K, Waugh R, Rokhsar DS, Muehlbauer GJ, Stein N, Gundlach H, Zytnicki M, Jamilloux V, Quesneville H, Wicker T, Faccioli P, Colaiacovo M, Stanca AM, Budak H, Cattivelli L, Glover N, Pingault L, Paux E, Sharma S, Appels R, Bellgard M, Chapman B, Nussbaumer T, Bader KC, Rimbert H, Wang S, Knox R, Kilian A, Alaux M, Alfama F, Couderc L, Guilhot N, Viseux C, Loaec M, Keller B, Praud S (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 345(6194). doi:10.1126/science.1251788

McCartney CA, Somers DJ, McCallum BD, Thomas JG, Humphreys DG, Menzies JG, Brown PD (2005) Microsatellite tagging of the leaf rust resistance gene *Lr16* on wheat chromosome 2BS. Mol Breeding 15:329–337

Mohler V, Lukman R, Ortiz-Islas S, William M, Worland AJ, Van Beem J, Wenzel G (2004) Genetic and physical mapping of photoperiod insensitive gene *Ppd-B1* in common wheat. Euphytica 138:33–40

Ouyang SH, Zhang D, Han J, Zhao XJ, Cui Y, Song W, Huo NX, Liang Y, Xie JZ, Wang ZZ, Wu QH, Chen YX, Lu P, Zhang DY, Wang LL, Sun H, Yang TM, Keeble-Gagnere G, Appels R, Dolezel J, Ling HQ, Luo MC, Gu YQ, Sun QX, Liu ZY (2014) Fine physical and genetic mapping of powdery mildew resistance gene *MlIW172* originating from wild emmer (*Triticum dicoccoides*). PLoS One 9(6):e100160

Richards RA, Rawson HM, Johnson DA (1986) Glaucousness in wheat: its development and effect on water-use efficiency, gas exchange and photosynthetic tissue temperature. Aust J Plant Physiol 13:465–473

Rong JK, Millet E, Manisterski J, Feldman M (2000) A new powdery mildew resistance gene: introgression from wild emmer into common wheat and RFLP-based mapping. Euphytica 115:121–126

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley—Mendelian inheritance, chromosomal location, and population-dynamics. Proc Natl Acad Sci USA 81:8014–8018

Scarth R, Law CN (1983) The location of the photoperiod gene *Ppd2* and an additional genetic factor for ear-emergence time on chromosome 2B of wheat. Heredity 51:607–619

Schnurbusch T, Collins NC, Eastwood RF, Sutton T, Jefferies SP, Langridge P (2007) Fine mapping and targeted SNP survey using rice-wheat gene colinearity in the region of the *Bo1* boron toxicity tolerance locus of bread wheat. Theor Appl Genet 115:451–461

Singh RP, William HM, Huerta-Espino J, Crosby M (2003) Identification and mapping of gene *Yr31* for resistance to stripe rust in *Triticum aestivum* cultivar Pastor. In: Pogna NE, Romano N, Pogna EA, Galterio G (eds) Proceedings of the 10th International Wheat Genetics Symposium, Instituto Sperimentale per la Cerealcoltura, Roma, Italy 1: 411–413

Somers DJ, Isaac P, Edwards K (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Tsunewaki K (1962) Monosomic analysis of synthesized hexaploid wheats. Jap J Genet 37:155–168

Tsunewaki K (1966) Comparative gene analysis of common wheat and its ancestral species. 2. Waxiness growth habit and awnedness. Jap J Bot 19:175–254

Tsunewaki K, Ebana K (1999) Production of near-isogenic lines of common wheat for glaucousness and genetic basis of this trait clarified by their use. Genes Genet Syst 74:33–41

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

Wang J, Li WL, Wang W (2014a) Fine mapping and metabolic and physiological characterization of the glume glaucousness inhibitor locus *Iw3* derived from wild wheat. Theor Appl Genet 127:831–841

Wang ZZ, Cui Y, Chen YX, Zhang DY, Liang Y, Wu QH, Xie JZ, Ouyang SH, Li DL, Huang YL, Lu P, Wang GX, Yu MH, Zhou SH, Sun QX, Liu ZY (2014b) Comparative genetic mapping and genomic region collinearity analysis of the powdery mildew resistance gene *Pm41*. Theor Appl Genet 127:1741–1751

Wu HB, Qin JX, Han J, Zhao XJ, Ouyang SH, Liang Y, Zhang D, Wang ZZ, Wu QH, Xie JZ, Cui Y, Peng HR, Sun QX, Liu ZY (2013) Comparative high-resolution mapping of the wax inhibitors *Iw1* and *Iw2* in hexaploid wheat. PLoS One 8(12):e84691

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

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

Yoshiya K, Watanabe N, Kuboyama T (2011) Genetic mapping of the genes for non-glaucous phenotypes in tetraploid wheat. Euphytica 177:293–297

Zhang ZZ, Wang W, Li WL (2013) Genetic Interactions underlying the biosynthesis and inhibition of beta-diketones in wheat and their impact on glaucousness and cuticle permeability. PLoS One 8(1):e54129