Theor Appl Genet (2015) 128:1467–1479
DOI 10.1007/s00122-015-2518-9

ORIGINAL PAPER

Molecular characterization of lipoxygenase genes on chromosome 4BS in Chinese bread wheat (*Triticum aestivum* L.)

Fuyan Zhang[1] · Feng Chen[1,2,3] · Peipei Wu[1] · Ning Zhang[1] · Dangqun Cui[1,2,3]

Received: 26 November 2014 / Accepted: 11 April 2015 / Published online: 22 April 2015

Abstract

Key message **This study cloned two novel *TaLox* genes on chromosome of 4BS and developed a co-dominant marker, Lox-B23, in bread wheat that showed highly significant association with lipoxygenase activity.**

Abstract Lipoxygenase (Lox), a critical enzyme in the carotenoid biosynthetic pathway, significantly influences the color and processing quality of wheat-based products. Two novel Lox genes, designated *TaLox-B2* and *TaLox-B3*, were cloned on chromosome 4BS of Chinese bread wheat. The deduced amino acid sequence showed that both *TaLox-B2* and *TaLox-B3* genes encoded an 861-aa protein and possessed a lipoxygenase superfamily domain at the 170–838 interval. Two different *TaLox-B2* alleles, designated *TaLox-B2a* and *TaLox-B2b*, were subsequently discovered. A co-dominant marker, Lox-B23, was developed based on sequences of *TaLox-B2a*, *TaLox-B2b*, and *TaLox-B3* genes to precisely distinguish these three alleles in Chinese bread cultivars. Among five allelic combinations of Lox genes at *Lox-B1*, *Lox-B2*, and *Lox-B3* loci, wheat cultivars with *TaLox-B1a/TaLox-B2a/TaLox-B3a* combination exhibited the highest Lox activity, whereas those with *TaLox-B1a/TaLox-B2b/TaLox-B3b* combination significantly showed the lowest Lox activity. A RIL population was used to evaluate the influence of *TaLox-B3a* gene on Lox activity. Results showed that *TaLox-B3a* gene could significantly increase the Lox activity in bread wheat. Physical mapping indicated that both *TaLox-B2* and *TaLox-B3* genes were located on chromosome 4BS in bread wheat. This study provides useful information to further understand the molecular and genetic bases of Lox activity in bread wheat.

Communicated by I. D. Godwin.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-015-2518-9) contains supplementary material, which is available to authorized users.

✉ Feng Chen
chf0088@163.com

✉ Dangqun Cui
cdq62@sohu.com

Fuyan Zhang
zhangfuyan704@163.com

Peipei Wu
wupeiwozaiwujia@qq.com

Ning Zhang
zhangning88012@126.com

[1] Collaborative Innovation Center of Henan Grain Crops, Zhengzhou 450002, China

[2] National Key Laboratory of Wheat and Maize Crop Science, Zhengzhou 450002, China

[3] Agronomy College, Henan Agricultural University, Zhengzhou 450002, China

Introduction

Lipoxygenase (Lox) belongs to a large family of enzymes known as non-heme iron-containing dioxygenases; Lox is widely found in animals, plants, and fungi and is particularly abundant in seeds (Shibata et al. 1994; Porta and Rocha-Sosa 2002; Liavonchanka and Feussner 2006). Most Lox-generated metabolites from different reactions are collectively called oxylipins, which play important roles in the regulation of plant growth and development (Berg et al. 2006), senescence (Seltmann et al. 2010), wounding and stress response (Veronico et al. 2006), and plant defense (Wang et al. 2008). The Lox content in wheat grain is very low, but the reactive oxygen species produced during

enzymatic oxidation of fat not only can degrade the pigment, which influences the color of flour, noodles, steamed bread, and bread flour products (Manna et al. 1998; Loiseau et al. 2001), but also can effectively improve the nutritional and processing qualities of flour products (Shiiba et al. 1991; Trufanov et al. 2007; Permyakova et al. 2010; Permyakova and Trufanov 2011).

Flour color is a key quality trait for wheat and plays a critical role in determining end-use products and marketing (He et al. 2004; Fu et al. 2008). Flour contains abundant natural pigments (lutein and carotenoids), which significantly influence the color and nutrition of noodles and steamed bread flour products (Crawford et al. 2011). Previous studies showed that flour color is attributed to natural carotenoid pigments present in seeds and is affected by enzymes, such as Lox (Manna et al. 1998; Loiseau et al. 2001; Trufanov et al. 2007), phytoene synthase (Psy) (He et al. 2009; Crawford et al. 2011), and polyphenol oxidase (Baik et al. 1995; Chang et al. 2007) during the processing of end-use products.

Flour pigment is mainly degraded during milling and processing of flour products. Milling determines mechanical loss of pigments mainly located at the external layer of the kernel with a low quantity for pasta processing, in which the maximal pigment degradation by Lox activity occurs (Borrelli et al. 1999). Lox is one of the most important enzymes contributing to semolina bleaching (Manna et al. 1998; Borrelli et al. 1999). Wheat cultivars with low Lox activity may have high nutritional value (Žilić et al. 2010). The treated flour with Lox isozyme showed shorter dough development time and less resistance after peak time on a mixogram than the control flour without enzyme treatment (Shiiba et al. 1991). The negative correlation between specific Lox activity and dough extensibility explains the ambivalent role of this enzyme in gluten and dough quality (Permyakova et al. 2010). Lox activity is negatively correlated with flour strength (Trufanov et al. 2007). This finding proved that Lox activity not only affects the color of flour and flour products, but also is closely related with gluten strength and dough rheological properties. Lox is one of the most important factors affecting the storage characteristics of bread wheat. Leenhardt et al. (2006) found that low Lox activity or Lox gene deletions can effectively decrease lipid oxidation, thereby reducing grain oxidative deterioration and extending the storage period. Consequently, low Lox activity in bread wheat cultivars of grain or flour increases storage time, thereby enhancing the commercial value (Leenhardt et al. 2006; Borrelli et al. 2008).

Given the rapid progress of modern molecular and biological techniques, many Lox genes have been mapped and cloned in cereal crops, including barley (*Hordeum vulgare* L.) (van Mechelen et al. 1999), durum wheat (*Triticum turgidum* L.) (Carrera et al. 2007; Garbus et al. 2009; Verlotta et al. 2010), and bread wheat (*Triticum aestivum* L.) (Feng et al. 2010, 2012; Geng et al. 2012; Garbus et al. 2013). To date, partial or complete sequences of eight Lox genes, namely, *Lpx-A3* (DQ474242 and DQ474244), *Lpx-A1_like* (FJ518909), *Lpx-B1.1* (DQ474240), *Lpx-B1.2* (DQ474241), *Lpx-B1.3* (HM126469), *Lpx-B3* (DQ474243), *Lpx-A2* (DQ448002), and *Lpx-B2* (DQ448001), have been cloned from durum wheat and are located on chromosomes 4A, 4B, 5A, and 5B (Hessler et al. 2002; Carrera et al. 2007; Zhang et al. 2008 Garbus et al. 2009; Verlotta et al. 2010). Three alleles have also been identified for *Lpx-B1.1*, *Lpx-B1.1a* (HM126466), *Lpx-B1.1b* (HM126468), and *Lpx-B1.1c* (HM126470) (Verlotta et al. 2010). Four *Lpx* sequences have been identified and annotated in *T. aestivum* genome, including *Lpx* pseudogene in the A genome (*Lpx-A1_like*; KC679303), *LpxB1.1*^{a} (KC679300) and *LpxB1.2* (KC679301) genes in the B genome, and *Lpx-D1* (KC679302) in the D genome (Garbus et al. 2009, 2013). Feng et al. (2010, 2012) cloned the gDNA or cDNA sequences of three *Lox* genes, i.e., *TaLox1* (GU167920/GQ166692), *TaLox2* (GU167921/GQ166691), and *TaLox3* (HQ913602). Amplified gDNA sequences of *TaLox-B1* from bread wheat cultivars with diverse grain Lox activities revealed the presence of two alleles, i.e., *TaLox-B1a* (HQ406779) and *TaLox-B1b* (HQ406780); one single-nucleotide polymorphism (SNP) has been detected in the third exon of *TaLox-B1a* and *TaLox-B1b* (Geng et al. 2012).

Although several Lox genes have been cloned, bread wheat contains a large Lox gene family and requires more Lox genes to precisely improve wheat quality in terms of Lox activity (Hart and Langston. 1977; Li et al. 1999). The Lox activity of bread wheat is also mainly influenced by Lox genes on chromosome 4 (Hart and Langston. 1977; Li et al. 1999; Verlotta et al. 2010; Garbus et al. 2013). Therefore, the current study focused on cloning Lox genes on chromosome 4B and identified the molecular characterization in bread wheat, as well as evaluated the association of these genes with Lox activity in Chinese bread wheat cultivars.

Materials and methods

Plant materials

Four Chinese winter wheat cultivars (Yunong 201, Zhoumai 18, Zhengmai 004, and Gaocheng 8901) were used for cloning *TaLox* genes in this study. The wheat plants were grown in a greenhouse under the condition of 16 h light at 25 °C (day) and 8 h dark at 22 °C (night). The root, stem, and leaf of each cultivar were collected at three-leaf seedlings and stored at −80 °C until analysis. The seedlings of Yunong 201 at the three-leaf stage was exposed at four

different stress treatments of 4 °C low temperature, 42 °C high temperature, 100 mmol L^{-1} NaCl, and 20 % PEG 6000 treatments for 0, 1, 3, 6, 12, and 24 h, consecutively. A whole set of Chinese Spring nullisomic–tetrasomic lines and ditelosomic lines as well as a F_{10} RIL population (UC 1110 × PI 610750) composed of 187 lines, kindly provided by Prof. Jorge Dubcovsky in University of California, Davis, were used to map the *TaLox* genes on chromosome location in bread wheat. A $F_{4:5}$ RIL population encompassing 110 lines, derived from Zhoumai 16 (with relatively high Lox activity) × Xianyang 83104 (with relatively low Lox activity), was used to examine the association of allelic variation of Lox genes on 4BS with Lox activity. A total of 136 bread wheat cultivars mainly from the wheat-producing area of China were used to evaluate the association of allelic variation of Lox genes on 4BS with Lox activity.

Field trials

During the 2011–2012 and 2012–2013 cropping seasons, the $F_{4:5}$ RIL population encompassing 110 lines and their parents were sown at the Zhengzhou Scientific Research and Education Center of Henan Agricultural University, China, and 136 Chinese winter wheat cultivars and advanced lines (Supplemental 1) were sown at Anyang (N36.1°, E114.5°), Zhengzhou (N34.9°, E113.6°), and Zhumadian (N32.9°, E114.1°) in randomized complete blocks with two replicates. Each plot comprised two 2 m rows spaced 25 cm apart with 75 plants in each row. Test plots were managed according to local practices. The field trials were kept free of weeds and diseases by broad-range herbicides and fungicides. After harvest, grain samples were cleaned. Falling number tests indicated that the plants were free of sprouting damage (data not shown).

Assays of grain Lox activity and colorimeter parameters

The total Lox activity in the mature seeds of the wheat cultivar was measured by the accumulation of hydrogen peroxide in the Lox reaction as described by Surry (1964). Extraction was performed as described by Geng et al. (2011) and Feng et al. (2012) with minor modifications. A single mature seed was crushed and suspended in 500 μL of cold extraction buffer (40 mM Na phosphate, pH 6.8). The homogenate was incubated on ice for 1 h with vortexing every 20 min. After centrifugation at 4 °C and 12,000 rpm for 10 min, the supernatant containing Lox was used for subsequent assay of Lox activity. The protein concentration of the enzyme solution was determined using the ELx808 model enzyme-labeled instrument (BioTek Instruments Inc., USA) with bovine serum albumin as standard. Negative controls of the samples were prepared by inactivating the crude enzymes by heat treatment at 100 °C for 10 min. The crude enzymes were incubated at 30 °C for 3 min in a cocktail of 2 mM linoleic acid (99 %), 0.05 % Tween 20, and 1 M NaOH. The reaction was stopped by adding an equal volume of 0.1 M NaOH. Lox activity was determined by measuring the conjugated diene absorption at 234 nm with an ultraviolet (UV)–visible spectrophotometer [UV-2600, Unico (Shanghai) Instruments Co., Ltd., Shanghai, China]. A 1-cm-thick quartz cuvette was used to measure the solution. One unit of Lox activity was defined as an increase in absorbance at 234 nm/min/g of whole wheat meal under assay conditions. For each data, Lox activity was determined in duplicate extracts of a single seed meal with parallel spectrophotometric measurements and mean values. If the coefficient of variation was >10 % for spectrophotometric assay of Lox activity in the duplicates, the test was repeated.

Wheat grains of each cultivar were milled into flour in a Chopin CD1 laboratory mill (Chopin Technologies, Paris, France) according to the method of Chen et al. (2013a). Flour color parameters of 136 Chinese wheat cultivars surveyed were measured by Colorimeter CR-410 (Konica Minolta Holdings, Inc.), i.e., L^* (lightness), a^* (redness) and b^* (yellowness).

Cloning and sequence analyses of *TaLox-B2* and *TaLox-B3* genes

The cDNA sequences of bread wheat *TaLox1* (GQ166692) and *TaLox2* (GQ166691) genes were used for BLAST search against the wheat expressed sequence tag (EST) database in GenBank. All wheat ESTs sharing high similarity with the reference gene were subjected to overlapping sequence assembly (E value $<10^{-50}$, score >500 bp, and identity >95 %; NCBI 2012). These ESTs were aligned, and the 5′ or 3′ end nucleotide sequences were compared by DNAMAN Version 6.0 software (http://www.softlandsl.com/free/dnaman+6+full.html). Subsequently, 20 primer sets were designed in the different conserved regions of the alignment sequences to obtain new sequences of *TaLox* genes in bread wheat. Finally, one primer set of Lox-P1 successfully amplified a full-length Lox cDNA (Table 1). Based on the Lox cDNA sequences cloned, three chromosome-specific primer sets of Lox-P2, Lox-P3, and Lox-P4 (Table 1) were redesigned to amplify the full-length gDNA sequence. All primers were designed by Premier Primer 5.0 software (http://www.primer-premier.findmysoft.com/) and synthesized by Sangon Biotech (Shanghai) Co., Ltd.

Genomic DNA was isolated from seedlings following a method modified from that of Lagudah et al. (1991) and Chen et al. (2012). PCR amplifications were performed in a BioRad-S1000 or ABI 9700 thermal cycler. Reactions were conducted in a 20 μL volume containing 50 ng of genomic

Table 1 Primer sets used for identification of *TaLox-B2* and *TaLox-B3* genes in this study

Primer	Primer sequences (5′–3′)	Annealing temp (°C)	Size of PCR fragment (bp)	References
Lox-P1	Forward: ATGATACTGGGCGGGCTCGT	60	2586	In this work
	Reverse: TCAGATGGAGATGCTGTTGGG			
Lox-P2	Forward: GCGTCACCTGCCAGCTTAT	66	1596	In this work
	Reverse: GAAGTCGGAGGTCTTGAGGTG			
Lox-P3	Forward: TACCTGCCGAGCCAGATGCC	66	1551	In this work
	Reverse: GTGCCACCCAGAGTCGTTGA			
Lox-P4	Forward: TCATCTACGCCACCAGGACC	64	1309	In this work
	Reverse: TCGCTGAACCGCTTGAACAC			
TaLox-P1	Forward: GAGGAAGAACGTGCTGGACC	60	1703	In this work
	Reverse: AGGATGCCCTGCGTGATGG			
TaLox-P2	Forward: CTGGGCTACTCCATCAAGGC	58	1422	In this work
	Reverse: GGGAACACCGTCATCTCAAAG			
TaLox-P3	Forward: CGGTGATGGAGCCGTTCGTGA	64	1241	In this work
	Reverse: ATGGAGATGCTGTTGGGGATG			
Lox-P5	Forward: AACAACCTGCCCGGCAACTTC	64	179	In this work
	Reverse: CACACCCATCCTTCGACGCT			
Lox-P6	Forward: CTCATGGATCTACCCCACCTC	64	193	In this work
	Reverse: GTCGTTGTAGACGTCGTAGCG			
Wac	Forward: GTTCCAATCTATGAGGGATACACGC	64	422	Chen et al. (2013a, b)
	Reverse: GAACCTCCACTGAGAACAACATTACC			
Lox16	Forward: CCATGACCTGATCCTTCCCTT	60	489	Geng et al. (2012)
	Reverse: GCGCGGATAGGGGTGGT			
Lox18	Forward: ACGATGTGAGTTGTGACTTGTGA	60	791	Geng et al. (2012)
	Reverse: GCGCGGATAGGGGTGC			
Lox-B23	Forward: TTCCCGCTCCAGCTCATCAA	64	788/677/660	In this work
	Reverse: AGGGCCTGCATGCAACAAGT			

DNA, 5 pmol of each primer, 200 μmol of each dNTP, 1× reaction buffer (50 mmol KCl, 10 mmol Tris–HCl, 1.5 mmol $MgCl_2$, pH 8.4), and 1 unit of *Taq* DNA polymerase (TIANGEN Biotech Co., Ltd., Beijing). The high-fidelity *Taq* DNA polymerase (Takara Bio, Inc.) was used to generate amplification fragments for cloning *TaLox* genes. Reaction conditions were 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 60 °C to 66 °C (specific annealing temperature of each primer pair is presented in Table 1) for 45 s, 72 °C for 90 s, and a final extension of 10 min at 72 °C. PCR products were separated and analyzed on 1.0–1.5 % (w/v) agarose gels, stained with ethidium bromide, and visualized with UV light. Targeted fragments with expected sizes based on the primer-binding sites in the DNA sequence were purified from the gels using Quick DNA extraction kit [TaKaRa Biotechnology (Dalian) Co., Ltd.]. Purified products were ligated into pMD19-T vector [TaKaRa Biotechnology (Dalian) Co., Ltd.] and transformed into cells of the *Escherichia coli* DH-5α strain. Plasmids containing targeted fragments were extracted by Plasmid Rapid Isolation Kit (Beijing CoWin Biotech Co., Ltd.) and sequenced from 10 clones for each sample by Sangon Biotech (Shanghai) Co., Ltd.

Complete multiple alignments of sequences and translations of nucleotides into amino acid sequences were performed by DNAMAN Version 6.0 software (http://www.softlandsl.com/free/dnaman+6+full.html). Reliability of sequencing results was verified by examining the sequence chromatograms using Chromas Version 1.4.5 and FinchTV 1.5.0 (http://www.geospiza.com/Products/finchtv.shtml). Phylogenetic trees of Lox genes were obtained by MEGA 6.06 software (http://www.megasoftware.net/history.php). Neighbor-joining tree was bootstrapped using five distance methods: (1) observed divergence, (2) Kimura, (3) Jukes–Cantor, (4) maximum likelihood with Poisson, and (5) maximum likelihood with Hasegawa–Kishino–Ya.

Quantitative RT-PCR analysis of *TaLox-B2* and *TaLox-B3* genes

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA), and first-strand cDNA was synthesized

using the SuperScript™ First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Gene-specific primers Lox-P5 and Lox-P6 were designed to examine the expression levels of *TaLox-B2* and *TaLox-B3* genes. Wheat *β-actin* gene (Table 1) was selected as internal control with the primer Wac (Chen et al. 2013b).

The expression profiles of *TaLox-B2* and *TaLox-B3* genes were measured using the cDNA samples from different tissues (root, stem, and leaf) of the three wheat cultivars (Zhoumai 18, Zhengmai 004, Gaocheng 8901) as well as from seeds of different developmental stages of cultivar Aikang 58. Quantitative RT-PCR was performed using the Bio-Rad iQ5 Sequence Detection System (Applied Biosynthesis, CA, USA) with the SYBR *Premix Ex Taq* II [TaKaRa Biotechnology (Dalian) Co., Ltd.]. The PCR conditions consisted of an initial denaturation step for 2 min at 94 °C, followed by 40 cycles of 10 s at 95 °C, 10 s at 58 °C, 20 s at 72 °C, and a final extension of 10 min at 72 °C. Wheat *β-actin* gene was selected as the internal control. The $2^{-\triangle\triangle CT}$ method was used to normalize and calibrate transcript values relative to the endogenous *β-actin* control. Six independent samples with triplicate repeats were analyzed.

Statistical analysis

For the 136 Chinese wheat cultivars and 110 $F_{4:5}$ RIL population, the Lox activity of each genotype was measured in cropping seasons and averaged to examine the association between Lox activity and newly developed markers. The Lox activities of all materials were measured with two replications and averaged for statistical analyses. Analysis of variance was conducted by PROC MIXED in the Statistical Analysis System (SAS Institute 2000) with genotype classes as categorical variables to derive the mean Lox activity for each class and test the significance level for the two classes. The differences in Lox activities among genotypes with different PCR band profiles (660, 677, or 788 bp) were tested by least significant range multiple comparisons.

Results

Discovery of *TaLox-B2* and *TaLox-B3* genes in bread wheat

Based on a set of gDNA or cDNA sequences of *Lox* genes of durum wheat (DQ474240, HM126467, and HM126469), bread wheat (HQ913602, GU167920, and GU167921), and barley (L37358, L37359, and L35931), a new cDNA sequence of *TaLox* gene with 2586 bp was generated with the amplification of primer set Lox-P1 (Table 1) in Chinese cultivar Yunong 201. The full-length gDNA sequence of this new *TaLox* gene was assembled with the overlapped fragments by three successful amplifications of primer sets Lox-P2, Lox-P3, and Lox-P4 (Table 1). Physical mapping by a set of nullisomic–tetrasomic lines and ditelosomic lines of Chinese Spring indicated that this new *TaLox* gene was located on the short arm of chromosome 4B in Chinese Spring (Fig. 1). Therefore, this new *TaLox* gene was designated as *TaLox-B2* because of the existence of *TaLox1* (designated as *TaLox-B1* gene in this study) on 4BS of bread wheat according to the nomenclature of McIntosh et al. (2007) and Geng et al.

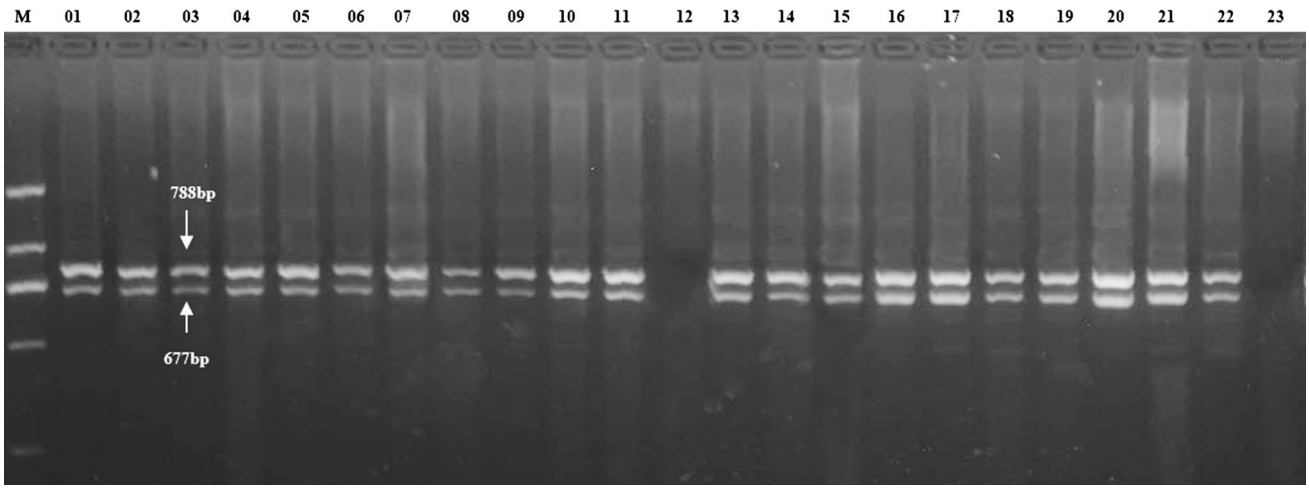

Fig. 1 Physical mapping of *TaLox-B2* and *TaLox-B3* genes by Lox-B23 marker in nullisomic–tetrasomic lines and ditelosomic lines in the Chinese Spring background. *M*: DL2000; *01*: Chinese Spring; *02*: N1A-T1D); *03*: N1B-T1A; *04*: N1D-T1B; *05*: N2A-T2D; *06*: N2B-T2A; *07*: N2D-T2B; *08*: N3A-T3D; *09*: N3B-T3D; *10*: N3D-T3A; *11*: N4A-T4D; *12*: N4B-T4A; *13*: N4D-T4B; *14*: N5A-T5B; *15*: N5B-T5A; *16*: N5D-T5B; *17*: N6A-T6B; *18*: Dt-4DL; *19*: Dt-4DS; *20*: Dt-4AL; 21: Dt-4AS; 22: Dt-4BS; *23*: Dt-4BL

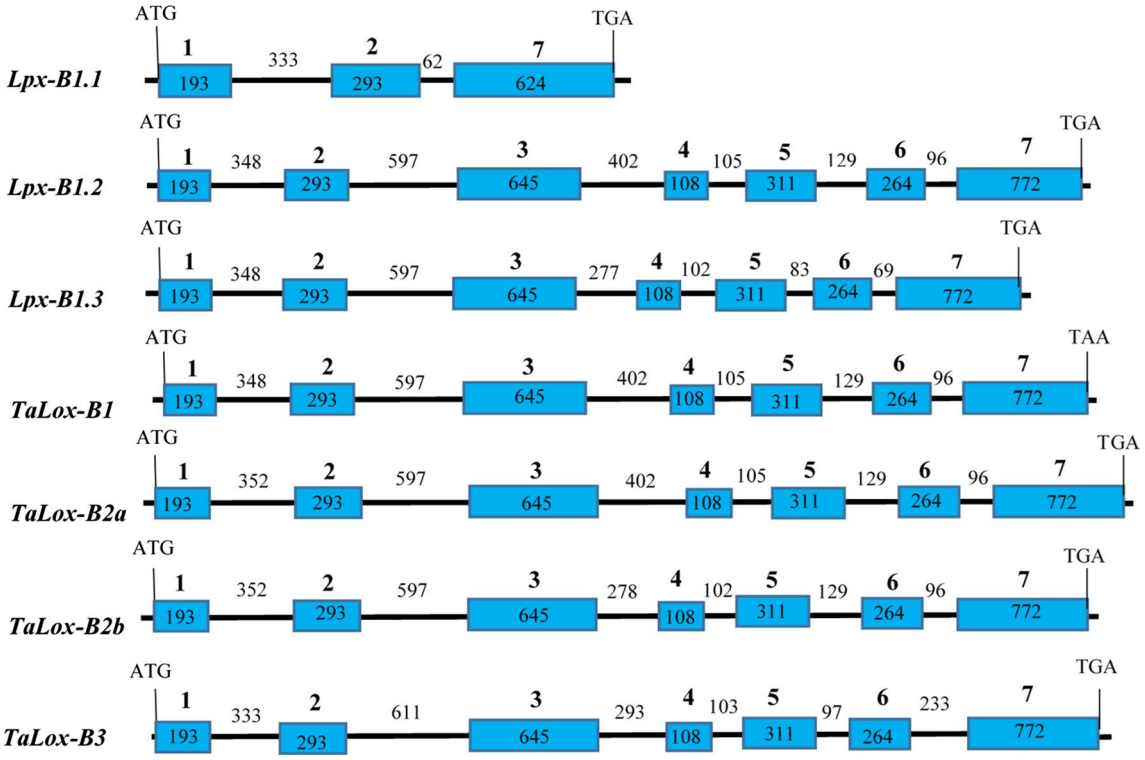

Fig. 2 Schematic representation of the structures of the *Lox* genes in durum and bread wheat. *Filled boxes* represent exons and *lines* represent introns

(2012). Sequence analysis of gDNA and cDNA of *TaLox-B2* gene indicated that this gene was composed of seven exons and six introns (Fig. 2). *TaLox-B2* gene with 4267 bp showed sequence similarities of 78.3 % with *TaLox1* (GU167920), 60.4 % with *TaLox2* (GU167921), and 60.9 % with *TaLox3* (HQ913602) at the DNA levels. The deduced amino acid sequence showed that *TaLox-B2* gene could encode an 861-aa protein and contained a Lox superfamily domain at the 170–838 interval as predicted using Pfam 27.0 software (http://www.pfam.xfam.org/).

However, when the primer set of Lox-P3 was used to amplify the *TaLox-B2* gene, two fragments (approximately 1500 and 1400 bp) with different sizes could be obtained simultaneously in the Chinese cultivar Yunong 201. Sequences of both fragments after ligation with T-Easy vector indicated that the approximately 1500-bp fragment belonged to the *TaLox-B2* gene, whereas the approximately 1400-bp fragment did not belong to any known *TaLox* genes. A new *TaLox* gene was also generated by sequencing several subclones encompassing overlapped sequences amplified with primer sets TaLox-P1, TaLox-P2, and TaLox-P3. The physical mapping by a set of nullisomic–tetrasomic lines and ditelosomic lines of Chinese Spring indicated that this new *TaLox* gene was also located on the short arm of chromosome 4B (Fig. 1). Therefore, this new *TaLox* gene was designated as *TaLox-B3* because of the existence of *TaLox-B1* and *TaLox-B2* genes according to the nomenclature of McIntosh et al. (2007) and Geng et al. (2012). Linkage analysis further showed that *TaLox-B3* gene was close to SSR marker *Barc20* (GrainGenes breakpoint interval report: 4BS8-0.57-0.81) and was located on chromosome 4BS (Fig. 3). Sequence analysis of gDNA and cDNA of *TaLox-B3* gene indicated that this gene consists of seven exons and six introns (Fig. 2). *TaLox-B3* gene with 4246 bp showed sequence similarities of 79.3 % with *TaLox1*, 62.1 % with *TaLox2*, 62.5 % with *TaLox3*, and 84.8 % with *TaLox-B2* at the DNA levels. However, the deduced amino acid sequence indicated that the *TaLox-B3* gene showed sequence similarities of 97.8 % with the *TaLox-B2* gene and encoded a protein of the same size (861 aa), which also contained a domain of lipoxygenase superfamily at the same interval with *TaLox-B2* gene (170–838).

Sequence blast of *TaLox-B1*, *TaLox-B2* and *TaLox-B3* genes in URGI database (http://www.wheat-urgi.versailles.inra.fr/) indicated that they were identical to three different contigs, i.e., IWGSC_chr4BS_ab_k71_contigs_longerthan_200_4957383 (*TaLox-B1* gene), IWGSC_chr4BS_ab_k71_contigs_longerthan_200_4963368 (*TaLox-B2* gene) and IWGSC_chr4BS_ab_k71_contigs_longerthan_200_4959255 (*TaLox-B3* gene), respectively. It suggested that *TaLox-B1*, *TaLox-B2* and *TaLox-B3* genes were not allelic and belonged to different genes.

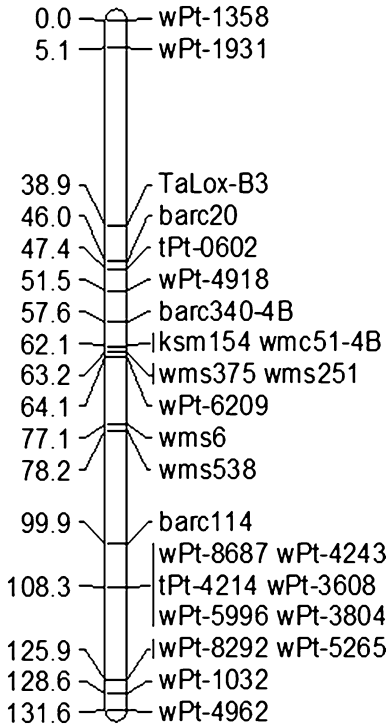

Fig. 3 Linkage map constructed with *TaLox-B3*, SSR markers and DArT markers on the chromosome 4B in the RIL population derived from UC 1110/PI 610750

Molecular characterizations of *TaLox-B2* and *TaLox-B3* genes in Chinese bread wheat cultivars

Furthermore, when primer set Lox-P3 was used to amplify *TaLox-B* gene, three different types of fragments appeared in various Chinese wheat cultivars: type I with two fragments of approximately 1500 and 1400 bp in Yunong 201 and Chinese Spring, type II with only an approximately 1500 bp fragment in Gaocheng 8901, and type III with only an approximately 1400 bp fragment in Zhengmai 004. Sequencing results indicated that the approximately 1500-bp fragment of Gaocheng 8901 exhibited 100 % identity with approximately 1500-bp fragment of Yunong 201 and Chinese Spring. The approximately 1500-bp fragment of type II belonged to the *TaLox-B2* gene. However, sequencing results of the approximately 1400-bp fragment of type III indicated that a new *TaLox-B2* allele was discovered in Zhengmai 004 after the complete alignment of this sequence with the *TaLox-B2* and *TaLox-B3* genes. The full-length sequence of this new *TaLox-B2* allele was obtained by assembling overlapped sequences of subcloned fragments amplified with primer sets Lox-P1 to Lox-P4. This new *TaLox-B2* allele in Zhengmai 004 was designated as *TaLox-B2b*, and the *TaLox-B2* allele in Yunong 201 was designated as *TaLox-B2a* according to the nomenclature of McIntosh et al. (2007) and Geng et al. (2012). Compared with the *TaLox-B2a* allele (Fig. 4), the *TaLox-B2b* allele exhibited two deletions (137 bp in the third intron and 3 bp in the fourth intron) and a 13-bp insertion in the third intron, as well as several SNPs. Complete alignment of *TaLox-B2b* allele with other *TaLox* genes of bread wheat indicated that *TaLox-B2b* allele showed sequence similarities of 99.4 % with *TaLox-B2a* allele, 85.1 % with *TaLox-B3*, 80.3 % with *TaLox1*, 62.0 % with *TaLox2*, and 61.6 % with *TaLox3* at the DNA levels (Supplemental 2). A polygenetic tree of different lipoxygenase genes from tetraploid and hexaploid wheat were shown in Supplemental 3.

Based on the sequences of *TaLox-B2a*, *TaLox-B2b*, and *TaLox-B3* genes, a new marker Lox-B23 (Table 1) was developed to precisely distinguish the differences of *TaLox-B2a*, *TaLox-B2b*, and *TaLox-B3* in Chinese bread wheat cultivars. The marker Lox-B23 could simultaneously generate two different expected fragments of 788 and 677 bp sizes in Yunong 201 or Zhoumai 18 with both *TaLox-B2a* and *TaLox-B3* genes, including only one expected 788 bp fragment in Gaocheng 8901 with *TaLox-B2a* allele and one expected 660 bp fragment in Zhengmai 004 with *TaLox-B2b* allele (Fig. 5). Sequencing results confirmed the reliability of the marker Lox-B23. A total of 136 Chinese wheat cultivars were identified with allelic variations of the *TaLox-B2* and *TaLox-B3* genes. The results indicated that 122 out of 136 Chinese wheat cultivars contained *TaLox-B2a* allele, and the remaining 14 cultivars included *TaLox-B2b* allele at the *TaLox-B2* locus (Supplemental 1). Up to 85 out of 136 Chinese wheat cultivars contained *TaLox-B3* gene, and the remaining 51 cultivars lacked this gene at the *TaLox-B3* locus. This finding suggested that the wheat cultivars with *TaLox-B2a* allele and *TaLox-B3* gene at the *TaLox-B2* and *TaLox-B3* loci were predominant in the surveyed Chinese wheat cultivars. The *TaLox-B3* gene was presented only in some Chinese wheat cultivars. The cultivars with *TaLox-B3* were designated as *TaLox-B3a* allele, and the cultivars without *TaLox-B3* were designated as *TaLox-B3b* allele. Notably, *TaLox-B2* gene existed in all of the cultivars surveyed, whereas all wheat cultivars with *TaLox-B3a* gene contained *TaLox-B2a* allele (Supplemental 1).

Associations of allelic variation of *TaLox-B* genes with Lox activity and colorimeter parameters in Chinese bread wheat

Analysis of variance indicated that the *TaLox-B* genes contributed significantly to variation of Lox activity in Chinese bread wheat cultivars and the influence of environment (Year, replicate and year × location, etc.) on Lox activity was also significant ($P < 0.05$) (Table 2). Analysis of

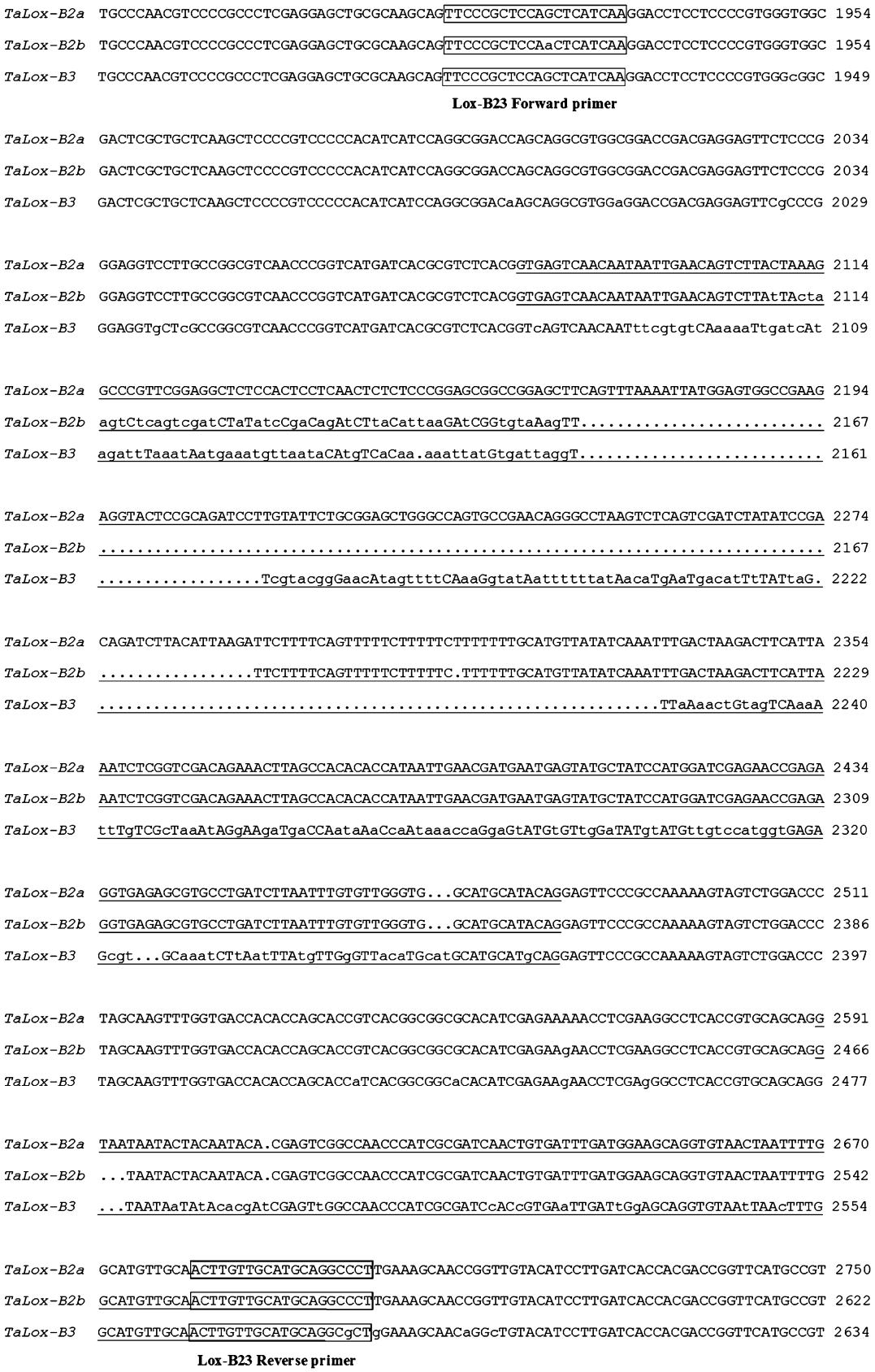

Fig. 4 Part alignment of DNA sequences of *TaLox-B2a*, *TaLox-B2b* and *TaLox-B3a* alleles in bread wheat. The sequences of the forward primers and complementary sequences of the reverse primers for Lox-B23 are *boxed* and the introns are *underlined*

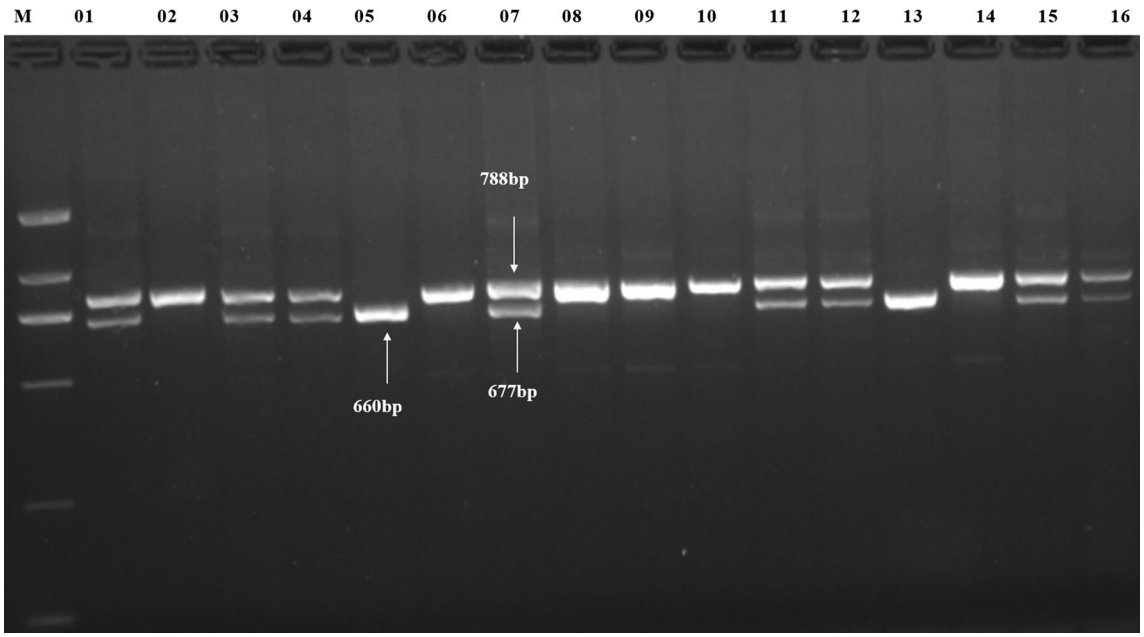

Fig. 5 Identification of *TaLox-B2* and *TaLox-B3* alleles by marker Lox-B23 in some Chinese wheat cultivars. *M*: DL2000; *01*: Zhoumai 18; *02*: Gaocheng 8901; *03*: Zhoumai 16; *04*: Yanzhan 4110; *05*: Zhengmai 004; *06*: Huaimai 19; *07*: Zhengmai 9023; *08*: Zhoumai 22; *09*: Shaan 160; *10*: Xianyang 83104; *11*: Aikang 58; *12*: Yunong 201; *13*: Xinmai 18; *14*: Yunong 211; *15*: Xinong 979; *16*: Yannong 15

Table 2 Mean-square values from ANOVA for Lox activity in Chinese bread wheat cultivars

Source of variation	*df.*	Mean square	Error	*F*
Genotype (*G*)	135	443.02**	242.47	1.83
Year (*Y*)	1	26438.68*	7307.63	3.62
Location (*L*)	2	1669.58	7185.13	0.23
Replicate (year × location) (*Y* × *L*)	6	216.05**	17.76	12.17
Genotype × year (*G* × *Y*)	135	225.23**	85.50	2.63
Genotype × location (*G* × *L*)	270	102.73*	85.50	1.20
Year × location (*Y* × *L*)	2	7167.89**	283.79	25.26
Genotype × year × location (*G* × *Y* × *L*)	270	85.50**	17.76	4.82

*, ** Indicate significant difference at the 0.05 and 0.01 levels, respectively

associations of allelic variation of *TaLox-B2* and *TaLox-B3* genes with Lox activity and colorimeter parameters indicated that wheat cultivars with *TaLox-B2a* allele exhibited a significantly higher Lox activity than that of cultivars with *TaLox-B2b* allele. Wheat cultivars with *TaLox-B3a* allele showed significantly higher Lox activity than that of cultivars with *TaLox-B3b* allele (Table 3). Wheat cultivars with *TaLox-B2b* allele showed the significantly highest *L**, the lowest *a** (absolute value) and *b** amongst cultivars with three different *TaLox-B* alleles. In the surveyed Chinese wheat cultivars, five allelic combinations were discovered at the *TaLox-B1*, *TaLox-B2*, and *TaLox-B3* loci, namely, *TaLox-B1a/TaLox-B2a/TaLox-B3a*, *TaLox-B1a/TaLox-B2a/TaLox-B3b*, *TaLox-B1a/TaLox-B2b/TaLox-B3b*, *TaLox-B1b/TaLox-B2a/TaLox-B3a*, and, *TaLox-B1b/TaLox-B2a/TaLox-B3b*. Further analysis of association of *TaLox-B*

Table 3 Association of *TaLox-B* alleles with Lox activities and colorimeter parameters in Chinese wheat cultivars surveyed

Marker	Allele	Sample no.	Frequency distribution (%)	Lox activity	Colorimeter parameter		
					*L**	*a**	*b**
Lox-B23	*TaLox-B2a/TaLox-B3a*	85	62.5	72.0 a	95.04 b	−0.49 a	8.68 a
	TaLox-B2a	37	27.2	68.5 b	94.97 b	−0.49 a	8.73 a
	TaLox-B2b	14	10.3	63.7 c	95.45 a	−0.37 b	7.77 b
Lox-16	*TaLox-B1a*	36	26.5	71.6 a			
Lox-18	*TaLox-B1b*	100	73.5	69.6 b			

Different letters after numbers indicate significant differences at the 0.05 level

Table 4 Comparison of Lox activities and colorimeter parameters of bread wheat cultivars with different *TaLox-B* allelic combinations

Allelic combination	Lox type	No. accessions	Lox activity (A_{234} $min^{-1}g^{-1}$)	Colorimeter parameter		
				*L**	*a**	*b**
TaLox-B1a/TaLox-B2a/TaLox-B3a	H	56	72.1 a	94.98 b	−0.48 a	8.65 a
TaLox-B1b/TaLox-B2a/TaLox-B3a	H	29	71.8 a	95.14 ab	−0.49 a	8.74 a
TaLox-B1b/TaLox-B2a/TaLox-B3b	MH	7	71.0 a	95.09 ab	−0.49 a	8.72 a
TaLox-B1a/TaLox-B2a/TaLox-B3b	ML	30	67.9 b	94.93 b	−0.49 a	8.82 a
TaLox-B1a/TaLox-B2b/TaLox-B3b	L	14	63.9 c	95.45 a	−0.37 b	7.77 b

Different letters after numbers indicate significant differences at the 0.05 level

H High Lox activity, *MH* moderately high Lox activity, *ML* moderately low Lox activity and *L* low Lox activity

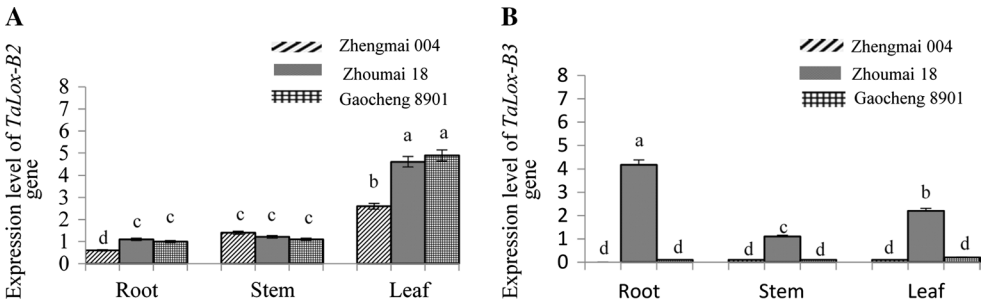

Fig. 6 Relative expression levels in the roots, stems, and leaves of three cultivars with different *TaLox-B* alleles. *Different letters* on the top of the *bars* indicated the significant difference at 0.05 probability level

allelic combination with Lox activity and colorimeter parameters indicated that wheat cultivars with the *TaLox-B1a/TaLox-B2a/TaLox-B3a* combination exhibited higher Lox activity than that of cultivars with the other four combinations (Table 4). By contrast, wheat cultivars with *TaLox-B1a/TaLox-B2b/TaLox-B3b* combination significantly showed the lowest Lox activity, *a** (absolute value) and *b** but the highest *L** among cultivars with the five combinations ($P < 0.05$, Table 4).

To determine the influence of *TaLox-B3* gene on Lox activity in bread wheat, a $F_{4:5}$ RIL population containing 110 lines derived from Xianyang 83104 with *TaLox-B1b/TaLox-B2a/TaLox-B3b* (61.7 A_{234} $min^{-1}g^{-1}$) and Zhoumai 16 with *TaLox-B1b/TaLox-B2a/TaLox-B3a* (80.0 A_{234} $min^{-1}g^{-1}$) was examined by the markers Lox16, Lox 18, and Lox-B23. The average Lox activity of each line in $F_{4:5}$ generations was used to analyze the association of *TaLox-B3* gene with Lox activity. The results indicated that out of the RIL population, 64 and 46 lines had *TaLox-B1b/TaLox-B2a/TaLox-B3a* and *TaLox-B1b/TaLox-B2a/TaLox-B3b* combinations, respectively. The average Lox activity of the wheat lines with *TaLox-B1b/TaLox-B2a/TaLox-B3a* (77.6 A_{234} $min^{-1}g^{-1}$) was significantly higher than that of lines with *TaLox-B1b/TaLox-B2a/TaLox-B3b* (68.1 A_{234} $min^{-1}g^{-1}$) ($P < 0.05$). This finding suggests that the existence of *TaLox-B3* gene could significantly increase the Lox activity in bread wheat.

Expression profile analysis of *TaLox-B2* and *TaLox-B3* genes

Expression profiles of the *TaLox-B2* and *TaLox-B3* genes were examined by RT-PCR in the wheat cultivars Zhoumai 18 (*TaLox-B2a/TaLox-B3a*), Zhengmai 004 (*TaLox-B2b/TaLox-B3b*), and Gaocheng 8901 (*TaLox-B2a/TaLox-B3b*) using gene-specific primers Lox-P5 and Lox-P6 (Table 1). RT-PCR results indicated that both *TaLox-B2* and *TaLox-B3* genes could express in the root, stem, and leaf of Zhoumai 18 with *TaLox-B2a/TaLox-B3a*, whereas *TaLox-B3* gene could not relatively express in those parts of Gaocheng 8901 and Zhengmai 004 with *TaLox-B3b* allele (Fig. 6a, b). Quantitative RT-PCR also showed that the relative expression level of *TaLox-B2* gene was significantly higher in the leaf than in the root and stem of Zhoumai 18, Zhengmai 004, and Gaocheng 8901 (Fig. 6a). Notably, the relative expression levels of *TaLox-B2a* (Zhoumai 18 and Gaocheng 8901) gene in the leaf were significantly higher than those of *TaLox-B2b* (Zhengmai 004). However, the relative expression levels of *TaLox-B2a* and *TaLox-B2b* genes in the root and stem were insignificantly different (Fig. 6a). The relative expression level of *TaLox-B3* gene was also significantly higher in the root than in the stem and leaf, with the lowest expression level in the stem of Zhoumai 18 with *TaLox-B3a* allele (Fig. 6b). Additionally, expression profiles of the *TaLox-B2* and *TaLox-B3* genes

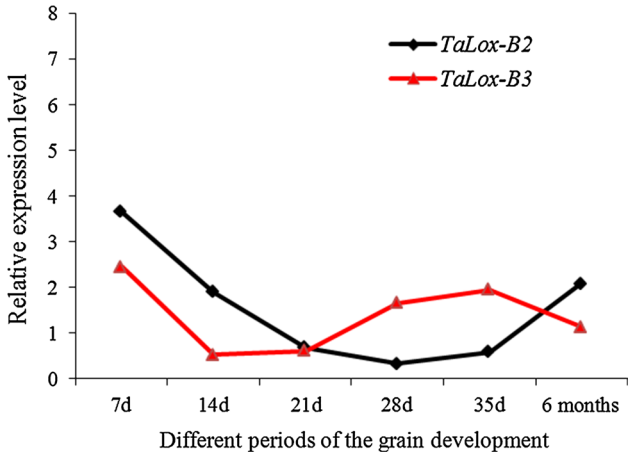

Fig. 7 Relative expression level of *TaLox-B2* and *TaLox-B3* genes in seeds of different developmental stages of Aikang 58 (*TaLox-B2a*/*TaLox-B3a*)

in seeds of the different developmental stages were shown in a Chinese current elite cultivar Aikang 58 (Fig. 7) with *TaLox-B2a* and *TaLox-B3a* genes.

Discussion

The role of Lox in wheat grain quality formation is largely understood. Leenhardt et al. (2006) and Borrelli et al. (2008) found that the low Lox activity or Lox gene deletions can effectively reduce the oxidation of lipids, thereby extending the storage period. This report proved that Lox is one of the most important factors affecting the storage characteristics of bread wheat. Consequently, Lox activity significantly affects wheat quality improvement and seed storage; however, Lox activity is controlled by a large Lox gene family (Hart and Langston. 1977; Li et al. 1999). Although several Lox genes have been discovered and cloned, the current study focused on cloning new Lox genes and obtaining two from these that are closely associated with Lox activity in bread wheat to precisely illustrate the molecular and genetic bases of Lox activity.

In wheat, previous studies have revealed that *Lpx1* and *Lpx3* are found on group 4 chromosomes, and *Lpx2* loci are located on group 5 chromosomes (Hart and Langston 1977; Li et al. 1999; Hessler et al. 2002; Carrera et al. 2007; Zhang et al. 2008; Garbus et al. 2009; Verlotta et al. 2010). *LoxA*, *LoxB*, and *LoxC* genes in the barley genome belong to a single copy of cloning, in which the gene sequence is relatively single (van Mechelen et al. 1995, 1999). In the A genome, a partially deleted copy of *Lpx-1* (*Lpx-A1-like*) was found, colocalizing within a 42-kb region with *Lpx-A3*. This finding confirmed that these two genes in both genomes were close to each other. The *Lpx-A1-like* gene is also found in 4A chromosomes of bread wheat (Garbus et al. 2013). *Lox* genes are located on the same chromosome segments in durum wheat and bread wheat and contained ortholog with barley (Li et al. 1999; Nachit et al. 2001). In barley, the Lox gene sequence is used as probe, and homologous cloning technology indicated multiple Lox gene cloning in wheat (Carrera et al. 2007; Feng et al. 2010, 2012). Seven Loxs, namely, *Lpx-A1_like*, *TaLox3*, *Lpx-B1.1*^{a}, *Lpx-B1.2*, *TaLox-B1*, *Lpx-D1*, and *TaLox1*, have been isolated and found on chromosomes 4A, 4B, and 4D in bread wheat (Feng et al. 2010, 2012; Geng et al. 2012; Garbus et al. 2013). This finding showed that Lox activity of bread wheat is mainly influenced by Lox genes on chromosome 4, whereas the two new *Lox* genes were located on chromosome 4B in the current study.

Grain Lox activity showed high heritability in bread wheat. Genotype and environment have great influence on Lox activity, and the genotype effect is higher than the effects of environment and genotype–environment interaction (Borrelli et al. 1999). Introns are integral elements of eukaryotic genomes that perform various important functions and actively participate in gene evolution (Fedorova and Fedorov 2003). In previous studies on wheat grain phytoene synthase (Psy) genes, divergence in the introns of *Psy* genes showed high associations with grain yellow pigment (YP) content (He et al. 2009; Crawford et al. 2011). Compared with *TaLox-B2a*, *TaLox-B2b* contained two deletions (137 bp in the third intron and 3 bp in the fourth intron), a 13-bp insertion in the third intron, and several SNPs, suggesting a similar molecular mechanism for intron splicing. Particularly, *TaLox-B2a* and *TaLox-B2b* showed the same exon size and common exon–intron structure with 99.4 and 100 % of identities in gDNA and cDNA sequences. *TaLox-B2a* and *TaLox-B2b* are highly conserved in structure and located parallel to the same chromosome location. This finding strongly implied that alternative splicing occurred in post-transcriptional modification of *TaLox-B2b*, resulting in a nonsense mutation generating a premature translation termination before the third exon; as a consequence, this mutation influences the expression of *TaLox-B2b*, leading to reduced grain Lox activity.

The Loxs are expressed in different organelles with different functions and are encoded by multiple gene families in higher plants (Porta and Rocha-Sosa 2002; Liavonchanka and Feussner 2006). Feng et al. (2012) found that Lox genes expressed different quantities in various tissues and organs of wheat. In the current study, qRT-PCR results indicated that both *TaLox-B2* (*TaLox-B2a* or *TaLox-B2b*) and *TaLox-B3* genes could be expressed in the root, stem, and leaf. The *TaLox-B2* gene was mainly expressed in the leaf, whereas the *TaLox-B3* gene was mainly expressed in the root.

Functional markers developed from polymorphic sites within genes that accurately discriminate counterpart alleles

at a locus should be used increasingly in wheat breeding (Bagge et al. 2007; Liu et al. 2012). To date, less functional markers for Lox genes have been reported in bread wheat. In the present study, a co-dominant functional marker, Lox-B23, for wheat grain Lox activity was developed and validated. The marker was highly relevant to Lox activity and can be used in wheat breeding programs aimed at improving the color of flour for various wheat-based end products.

Few cultivars with the 788-bp fragment still showed a low Lox activity, and the other cultivars with the 660-bp fragment exhibited a high Lox activity. These results may be attributed to multiple genes conditioning the Lox activity and environmental effects, particularly those on group 4 and 5 chromosomes (Hart and Langston 1977; Li et al. 1999; Feng et al. 2010, 2012; Garbus et al. 2013). Geng et al. (2012) developed dominant complementary functional markers Lox16 and Lox18, which can accurately discriminate the two alleles of *TaLox-B1*. Lox16 and Lox18 showed high associations with higher and lower Lox activities, respectively. Therefore, developing functional markers for the Lox gene on group 4 and 5 chromosomes and implementing them for wheat Lox evaluation in combination with Lox-B23 might be necessary for breeding wheat cultivars with low or high Lox activity.

In summary, we cloned two novel Lox genes on chromosome of 4BS, and identified two alleles of *TaLox-B2* gene in Chinese bread wheat cultivars, and analyzed the association of allelic variation of *TaLox* genes with Lox activity. Furthermore, quantitative real-time PCR indicated that *TaLox-B2* and *TaLox-B3* genes could express in various organs of wheat. This study could provide useful information to further understand the molecular and genetic bases of Lox activity in bread wheat.

Author contribution statement FC and DC designed this study. FZ and FC wrote the manuscript. FZ and PW performed experimental data for cloning of Lox genes and identification of Lox activity. FZ and NZ performed the qRT-PCR analyses. All authors reviewed the manuscript.

Acknowledgments This project was funded by the 973 projects (2014CB138105 and 2014CB160303), National Natural Science Foundation (31370031) and Program for New Century Excellent Talents in University (NCET-13-0776) of China.

Conflict of interest The authors declare that they have no conflict of interest.

References

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

Baik BK, Czuchajowska Z, Pomeranz Y (1995) Discoloration of dough for oriental noodles. Cereal Chem 72:198–205

Berg C, Hammarstrom S, Herbertsson H, Lindstrom E, Svensson AC, Soderstrom M, Tengvall P, Bengtsson T (2006) Platelet-induced growth of human fibroblasts is associated with an increased expression of 5-lipoxygenase. J Thromb Haemost 96:652–659

Borrelli GM, Troeeoli A, Fonzo ND, Fares C (1999) Durum wheat lipoxygenase activity and other quality parameters that affect pasta color. Cereal Chem 76:335–340

Borrelli GM, Deleonardis AM, Platani C, Troccoli A (2008) Distribution along durum wheat kernel of the components involved in semolina color. J Cereal Sci 48:494–502

Carrera A, Echenique V, Zhang W, Helguera M, Manthey F, Schrager A, Picca A, Cervigni G, Dubcovsky J (2007) A deletion at the *Lpx-B1* locus is associated with low lipoxygenase activity and improves pasta color in durum wheat (*Triticum turgidum spp. durum*). J Cereal Sci 45:67–77

Chang C, Zhang HP, Xu J, You MS, Li BY, Liu GT (2007) Variation in two PPO genes associated with polyphenol oxidase activity in seeds of common wheat. Euphytica 154:181–193

Chen F, Zhang FY, Xia XC, Dong ZD, Cui DQ (2012) Distribution of puroindoline alleles in bread wheat cultivars of the Yellow and Huai valley of China and discovery of a novel puroindoline a allele without PINA protein. Mol Breed 29:371–378

Chen F, Li HH, Li XN, Dong ZD, Zuo AH, Shang XL, Cui DQ (2013a) Alveograph and Mixolab parameters associated with *Puroindoline-D1* genes in Chinese winter wheats. J Sci Food Agricul 10:2541–2548

Chen F, Zhang FY, Li HH, Morris CF, Cao YY, Shang XL, Cui DQ (2013b) Allelic variation and distribution independence of *Puroindoline b-B2* variants and their association with grain texture in wheat. Mol Breed 32:399–409

Crawford AC, Stefanova K, Lambe W, McLean R, Wilson R, Barclay I, Francki MG (2011) Functional relationships of *phytoene synthase 1* alleles on chromosome 7A controlling flour colour variation in selected Australian wheat genotypes. Theor Appl Genet 123:95–108

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

Feng B, Dong ZY, Xu ZB, An XL, Qin HJ, Wu N, Wang DW, Wang T (2010) Molecular analysis of lipoxygenase (*LOX*) genes in common wheat and phylogenetic investigation of LOX proteins from model and crop plants. J Cereal Sci 52:387–394

Feng B, Dong ZY, Xu ZB, Wang DW, Wang T (2012) Molecular characterization of a novel type of lipoxygenase (*LOX*) gene from common wheat (*Triticum aestivum* L.). Mol Breed 30:113–124

Fu BX (2008) Asian noodles: history, classification, raw materials, and processing. Food Res Int 41:888–902

Garbus I, Carrera AD, Dubcovsky J, Echenique V (2009) Physical mapping of durum wheat lipoxygenase genes. J Cereal Sci 50:67–73

Garbus I, Soresi D, Romero J, Echenique V (2013) Identification, mapping and evolutionary course of wheat *lipoxygenase-1* genes located on the A genome. J Cereal Sci 58:298–304

Geng HW, Zhang Y, He ZH, Zhang LP, Appels R, Qu YY, Xia XC (2011) Molecular markers for tracking variation in lipoxygenase activity in wheat breeding. Mol Breed 28:117–126

Geng HW, Xia XC, Zhang LP, Qu YY, He ZH (2012) Development of functional markers for a lipoxygenase gene *TaLox-B1* on chromosome 4BS in common wheat. Crop Sci 52:568–576

Hart GE, Langston PJ (1977) Chromosome location and evolution of isozyme structural genes in hexaploid wheat. Heredity 39:263–277

He ZH, Yang J, Zhang Y, Quail KJ, Peña (2004) Pan bread and dry white Chinese noodle quality in Chinese winter wheats. Euphytica 139:257–267

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

Hessler TG, Thomson MJ, Benscher D, Nachit MM, Sorrells ME (2002) Association of a lipoxygenase locus, *Lpx-B1*, with variation in lipoxygenase activity in durum seeds. Crop Sci 42:1695–1700

Lagudah ES, Appels R, McNeil D (1991) The *Nor-D3* locus of *Triticum tauschii*: natural variation and genetic linkage to markers in chromosome 5. Genome 34:387–395

Leenhardt F, Lyana B, Rocka E, Boussardb A, Potusb J, Chanliaudc E, Remesy C (2006) Genetic variability of carotenoid concentration, and lipoxygenase and peroxidase activities among cultivated wheat species and bread wheat varieties. Europ J Agron 25:170–176

Li WL, Faris JD, Chittoor JM, Leach JE, Hulbert SH, Liu DJ, Chen PD, Gill BS (1999) Genomic mapping of defense response genes in wheat. Theor Appl Genet 98:226–233

Liavonchanka A, Feussner I (2006) Lipoxygenases: occurrence, functions and catalysis. J Plant Physiol 163:348–357

Liu YN, He ZH, Appels R, Xia XC (2012) Functional markers in wheat: current status and future prospects. Theor Appl Genet 125:1–10

Loiseau J, Vu BL, Macherel MH, Deunff YL (2001) Seed lipoxygeanses: occurrence and functions. Seed Sci Res 11:199–211

Manna F, Borrelli GM, Massardo DR, Wolf K, Alifano P, Giudice DL, Fonzo D, DiVerential N (1998) Differential expression of lipoxygenase genes among durum wheat cultivars. Cereal Res Commun 26:23–30

McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Anderson OD (2007) Catalogue of gene symbols for wheat: 2007 supplement. http://www.wheat.pw.usda.gov/ggpages/wgc/2007upd.html

Nachit MM, Elouafi I, Pagnotta MA, El Saleh A, Iacono E, Labhilili M, Asbati A, Azrak M, Hazzam H, Benscher D, Khairallah M, Ribaut JM, Tanzarella OA, Porceddu E, Sorrells ME (2001) Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. durum). Theor Appl Genet 102:177–186

Permyakova MD, Trufanov VA (2011) Effect of soybean lipoxygenase on baking properties of wheat flour. Appl Biochem Micro 47:315–320

Permyakova MD, Trufanov VA, Pshenichnikova TA, Ermakova MF (2010) Role of lipoxygenase in the determination of wheat grain quality. Appl Biochem Micro 46:87–92

Porta H, Rocha-Sosa M (2002) Plant lipoxygenases. Physiological and molecular features. Plant Physiol 130:15–21

Seltmann MA, Stingl NE, Lautenschlaeger JK, Krischke M, Mueller MJ, Berger S (2010) Differential impact of lipoxygenase 2 and jasmonates on natural and stress-induced senescence in Arabidopsis. Plant Physiol 152:1940–1950

Shibata D, Slusarenko A, Casey R, Hildebrand D, Bell E (1994) Lipoxygenases. Plant Mol Biol Rep 12:41–42

Shiiba K, Nengishi Y, Okada K, Nagao S (1991) Purification and characterization of lipoxygenase isozymes from wheat germ. Cereal Chem 68:115–122

Surry K (1964) Spectrophotometric method for determination of lipoxidase activity. Plant Physiol 39:65–70

Trufanov VA, Permyakova MD, Pshenichnikova TA, Ermakova MF, Davydov VA, Permyakov AV, Berezovskaya EV (2007) The effect of intercultivar substitution of wheat *Triticum aestivum* L. Chromosomes on lipoxygenase activity and its correlation with the technological properties of flour. Appl Biochem Micro 43:9l–97

van Mechelen JR, Smits M, Douma AC, Rouster J, Cameron-Mills V, Heidekamp F, Valk BE (1995) Primary structure of a lipoxygenase from barley grain as deduced from its cDNA sequence. Biochim Biophys Acta 1254:221–225

van Mechelen JR, Schuurink RC, Smits M, Graner A, Douma AC, Sedee NJA, Schmitt NF, Valk BE (1999) Molecular characterization of two lipoxygenases from barley. Plant Mol Biol 39:1283–1298

Verlotta A, Simone VD, Mastrangelo AM, Cattivelli L, Papa R, Trono D (2010) Insight into durum wheat *Lpx-B1*: a small gene family coding for the lipoxygenase responsible for carotenoid bleaching in mature grains. BMC Plant Biol 10:263

Veronico P, Giannino D, Melillo MT, Leone A, Reyes A, Kennedy MW, Bleve-Zacheo T (2006) A novel lipoxygenase in pea roots. Its function in wounding and biotic stress. Plant Physiol 141:1045–1055

Wang R, Shen WB, Liu LL, Jiang L, Liu YQ, Su N, Wan JM (2008) A novel lipoxygenase gene from developing rice seeds confers dual position specificity and responds to wounding and insect attack. Plant Mol Biol 66:401–414

Zhang W, Chao S, Manthey F, Chicaiza O, Brevis JC, Echenique V, Dubcovsky J (2008) QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. Theor Appl Genet 117:1361–1377

Žilić S, Dodig D, Šukalović VH-T, Maksimović M, Saratlić G, Škrbić B (2010) Bread and durum wheat compared for antioxidants contents, and lipoxygenase and peroxidase activities. Int J Food Sci Technol 45:1360–1367